SPATIAL ASPECTS OF BUMBLE BEE (*BOMBUS* SPP.: APIDAE) FORAGING IN FARM LANDSCAPES

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M.J. Schaffer

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Frontispiece. A large earth bumble bee worker *Bombus terrestris* (L.) foraging on lucerne flowers *Medicago sativa* L.. The bee's wings show one of the coloured fluorescent powders used during a mark-reobservation study in this thesis to trace movements by bumble bees in a commercial lucerne seed crop.

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SPATIAL ASPECTS OF BUMBLE BEE (*BOMBUS* SPP.: APIDAE)

FORAGING IN FARM LANDSCAPES

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Bumble bees (*Bombus* spp.: Apidae) are valuable pollinators of many crop and wildflower species. However, in some situations their potential is limited. Evaluation of, and management to improve bumble bee efficacy should include spatial information which is currently limited. Distance and direction determine the success of gene flow via pollen cross-over within and between plant populations at several scales. Studies of movement by bumble bees at large scales in semi-natural and intensively managed habitats are scarce. Few studies of bumble bee dispersal from the nest exist, particularly in relation to crops. At a small scale, directional rather than random movement between flowers has benefits for pollen flow. Results to date of directionality studies at small scales and their interpretation are inconsistent. The purpose of this thesis was to assess distances and directions moved by foraging bumble bees at a range of scales in two contrasting farm habitats in order to predict their pollination potential.

A novel method was developed to mark automatically all the occupants of nests of bumble bees *B. terrestris* (L.) placed around a lucerne seed crop *Medicago sativa* L. in New Zealand. Reobservation data from eight nests showed that of bumble bees which foraged within the crop, 81% travelled ≤ 50 m and 56% ≤ 20 m from their nest. Results should be interpreted with extreme caution because fewer than 1% of bumble bees marked at nests were reobserved in the crop. Because it was not established where the other 99% of the bumble bees went, foraging areas for nests could not be calculated as anticipated. Theories to explain the non-specificity of bumble bees to the crop include; resource depletion near nests, competition with honey bees in the crop, or an evolved strategy to disperse in order to minimise nest predation.

Lucerne flowers contained a significantly lower concentration of sugar in nectar, and significantly fewer pollen grains than did those of purple loosestrife *Lythrum salicaria* L., a species on which bumble bees appeared to forage in preference lucerne. The higher rewards offered by *L. salicaria* may have diverted bumble bees from the less-rewarding lucerne crop.

In a Norwegian meadow system, all foraging bouts by bumble bees *B. lucorum* (L.) within a patch of wood cranesbill flowers *Geranium sylvaticum* L. were random with respect to direction. This result is not consistent with predictions, based on optimal foraging theory, that movement should be directional to enable optimal pollen flow, and to avoid revisitation of just-emptied flowers by the pollinator. A medium-scale study of several bumble bee species moving between patches of northern wolfsbane *Aconitum septentrionale* Koelle in Norway revealed considerable loyalty by bumble bees to patches in which they were marked. In a different landscape-scale study (over 5 ha), several bumble bees exhibited a high degree of loyalty to areas in which they were marked (87% were reobserved ≤ 50 m from marking points). These restricted movement patterns are discussed in terms of potential pollen flow. Of 260 bumble bees marked, only five were recorded crossing between meadows, which could be a result of innate loyalty to small forage areas, an artefact of the sampling technique used, or forest boundaries acting as physical impediments to movement.

In the future, spatial data of the type collected in this thesis will aid in the management of bumble bee populations to achieve both commercial and conservation goals. Spatial data can be applied to predict the optimal placement of artificially-reared nests, predict suitable isolation distances for pure seed crops, and aid in the positioning of supplementary forage sources and nest-site refuges.

Keywords: Bumble bee, *Bombus*, foraging behaviour, pollination, movement, spatial approach, scale, distance, direction, foraging area, New Zealand, seed crop, Norway, meadow, pollen, nectar.

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Chapter 1

General introduction

Insect movement information has a wide range of applications. Knowledge of foraging, dispersal, and migration patterns of insects can enhance the effectiveness of pest control programmes, aid in the enhancement of beneficial insect populations, and contribute to the preservation of threatened insect and plant species which depend on each other for survival.

Studies concerning beneficial insects in farmland habitats to date have focused on the role of natural enemies in pest-related studies in intensive agricultural and horticultural systems. Most farmland types also contain beneficial insects other than natural enemies, and, in addition, plant or insect species of particular conservation value (Fry 1991). Pollinators are an example of valuable beneficial insects for which knowledge is limited in both semi-natural and intensively modified environments.

Pollination is a "keystone process" in maintaining biological diversity in semi-natural habitats and in providing sustainable productivity through seed yields of entomophilous crops in intensively-managed farmland systems (Kevan 1991). To understand how different farmland management regimes impact on the efficacy of pollinators, a knowledge of their foraging behaviour is required. In combination with the breeding system of the plant, pollen deposition, pollen carry-over and movement of seeds, the extent and success of gene flow is greatly dependent on movement patterns of the pollinator (Kearns & Inouye 1993).

Bumble bees *Bombus* spp. Latreille (Hymenoptera: Apidae) are important pollinators of many crop and wildflower species in farmland areas (Corbet *et al.* 1991). Two examples in which low seed yields have been attributed to limited pollination services, which bumble bees may have the potential to improve, are in lucerne seed crops (*Medicago sativa* L.; Leguminosae) in New Zealand (Donovan 1974; Dunbier *et al.* 1983) and in the decline of flower species of conservation value in abandoned traditional hay meadows in Norway (Fry 1993b). To improve the efficacy of bumble bees as pollinators in these situations through management, a knowledge

of the spatial aspects of bumble bee foraging behaviour will be advantageous. The literature so far is dominated with temporal aspects by bumble bee foraging behaviour.

Several studies of the movement of honey bees (*Apis mellifera.* L.; Apidae) exist. Although honey bees are relatively closely taxonomically related to bumble bees, the honey bee social system is much more highly developed than that of the primitively eusocial system of bumble bees (Michener 1974; Alford 1975). Critical differences between the *Apis* and *Bombus* genera include; the highly developed ability of honey bees to learn and communicate information regarding forage sources, a more rigid division of labour in honey bee colonies (Plowright & Laverty 1984), and morphological and physiological body differences which directly affect flight ability. Therefore, bumble bees should not necessarily be expected to exhibit the same patterns of movement which have been reported for honey bees, both at the individual or colony level.

Distance and direction are the basic parameters needed to describe movement patterns. Both have implications for the success of pollen flow. Increasing distance flown, and directional rather than random foraging patterns by the bee should theoretically increase the probability of out-crossing of genes within and between plant populations, increasing genetic vigour and maintaining population stability (Rasmussen & Brodsgaard 1992).

The foraging area of an individual bee or a whole colony is an important consideration in understanding use of space by whole colonies of pollinators, and has implications for the management of natural and artificial nest sites. Several authors have considered the space used by a bumble bee once it lands in a certain patch or area, but few have specifically considered foraging patterns of bumble bees in relation to their nest (but see Dramstad 1996b).

A framework in which to place insect movement studies has emerged from the landscape ecology discipline (see Forman & Godron 1986; Merriam 1988). An aim of landscape ecologists is to link patterns and processes operating at different scales. A hierarchy of scales ranging from small to large landscape studies provides a common structure to compare related studies and pinpoint where gaps in understanding exist. Pollination is an important process.

Pollinator movement studies and their relationship with plant distribution patterns (Dramstad 1996a), fit well within this framework, and are inreasingly relevant because many farm environments are undergoing drastic changes in the spatial arrangement of resources through habitat fragmentation (Rathcke & Jules 1993; Buchmann & Nabhan 1996).

The aim of this thesis was to quantify movement patterns of bumble bees at different spatial scales and in two field situations; a Norwegian meadow of conservation value, and a commercial seed crop in New Zealand. A secondary objective of this thesis was to relate the influence of plant rewards to foraging patterns exhibited by bumble bees.

Specific aims of this thesis were to:

1. In the Norwegian meadow site

- establish whether movement patterns by bumble bees within a flower patch were significantly directional, or random.
- establish visitation patterns between distinct patches of one flower species, to test whether bumble bees necessarily visited "nearest neighbour" patches.
- establish the variation of flight distances of bumble bees within a large scale meadow system from areas in which they were marked, as an indication of forage patch and area loyalty.
- assess whether bumble bees crossed between meadows within a forest matrix and if so, the extent of crossing.

2. In the New Zealand crop site

- develop a simple marking system to mark automatically all the occupants of bumble bee nests placed beside a crop, in order to trace bumble bee movement patterns within the crop, and hence estimate foraging areas of whole colonies.
- measure specificity of bumble bees from the introduced nests to the target crop, using time-lapse video recording.
- quantify variability in reproductive success between nests (numbers of workers produced), to account for any major differences in movement patterns calculated.

- compare pollen and nectar rewards offered by a crop species with those offered by a nearby weed species (which bumble bees appeared to prefer) in order to link the influence of plant rewards to movement patterns.

Movement patterns observed in this thesis are discussed in terms of behavioural strategies of bumble bees, resource distribution, competition with honey bees, climatic factors, and physical landscape features. Potential implications for plant success and implications for future management of bumble bee populations are discussed.

Thesis structure

The literature review and background chapter for this thesis (Chapter 2) is followed by three experimental chapters (Chapters 3, 4 and 5) after which a general discussion and conclusion chapter (Chapter 6) is given.

Chapter 2

Literature review and background to the study

2.1 Introduction

This chapter provides background information on bumble bees, pollination and movement studies relevant to this thesis. Section 2.2 describes the general biology and ecology of bumble bees and reviews aspects of their foraging biology in relation to pollination. Section 2.3 introduces the farmland sites used in experimental studies in Chapters 3 and 4. An overview of previous insect movement studies in general, then of bees in general is given in Section 2.4. In Section 2.4.2 previous movement studies of bumble bees are reviewed in relation to the spatial scales at which they were performed. Specific information on bumble bee movement from nests, and in relation to crops is considered. Section 2.5 provides background for Chapter 5 by outlining plant features which may influence bumble bee movement, focusing on pollen and nectar rewards.

2.2 Bumble bees (*Bombus* spp.) as pollinators

Section 2.2.1 describes the general biology and ecology of bumble bees. Section 2.2.2 outlines morphological and physiological adaptations, and behavioural strategies of foraging bumble bees which contribute to their pollinating abilities.

2.2.1 General biology and ecology of bumble bees

Taxonomy

The world bee fauna consists of approximately 20 000 species in the Superfamily Apoidea, which is divided into twelve families. "True bumble bees" belong to the Order Hymenoptera Suborder Apocrita, Family Apidae, Subfamily Bombinae, and Genus *Bombus* , which is further subdivided into several subgenera (see Appendix 2.1). There are approximately 300 *Bombus* species world-wide (Prys-Jones & Corbet 1991). New Zealand has four true bumble

bee species, all introduced (Donovan & Macfarlane 1984). Norway has 26 true bumble bee species and seven cuckoo bumble bee *Psithyrus* Lepeletier species (Løken 1985). "Cuckoo bees" are nest inquilines which live at the expense of true bumble bees by leaving eggs in their nests for the bumble bees to rear (Alford 1975). Cuckoo bees do not collect nectar and pollen for the colony as the worker caste of true bumble bees does, and all larvae develop directly into male or female reproductives (Prys-Jones & Corbet 1991). Details of taxonomic descriptions of the bumble bee species studied are given in Appendix 2.1, (following Donovan & Macfarlane 1984; Løken 1985; Prys-Jones & Corbet 1991).

Social status and division of labour

Bumble bees are primitively eusocial, i.e., the queen hibernates alone after the rest of the annual colony have died (Alford 1975). A division of labour exists within the colony between foragers and "nest" bees which is based on size (Alford 1975). Nest bees are small bees which carry out duties within the nest and generally do not forage. Workers' pollen and nectar collecting duties are flexible and change rapidly in response to colony protein and energy requirements. It has been shown that the extent of pollen collection is directly related to the amount of developing nest brood (Plowright & Laverty 1984). Communication by bumble bees is not as developed as that in honey bees (Michener 1974). Experiments by Brian (1954a) showed that bumble bees failed to return to a rich syrup resource which was offered to them, and failed to recruit new foragers through communication, which honey bees would have done. However, more recent work suggests that odour cues and acoustic signalling may occur between foragers both at the nest, and in the field (Plowright & Laverty 1984). Whether or not bumble bees communicate will have implications for their spatial foraging patterns, and therefore for management involving manipulation of resources.

Phenology of bumble bees in New Zealand and Norway

Bumble bee colonies are annual. Males and worker-caste bees die in autumn. Several new queens may be produced in each nest, and these leave the nest, mate and hibernate underground over winter. In spring the queens emerge and raise new colonies (Figure 2.1). Because New Zealand's climate is temperate, bumble bees of all castes can be seen year-round in certain localities (Donovan & Macfarlane 1984). Norway's seasons in contrast are very marked, with

extremely cold winters limiting bumble bee population abundance and duration and also that of forage sources. A key limitation to the success of bumble bee populations in New Zealand is the discontinuous nature of forage reserves throughout the year, which may not match the needs of the developing colony. Spring, when the new queens emerge, is regarded as a critical food-shortage time for bumble bees (Donovan & Macfarlane 1984).

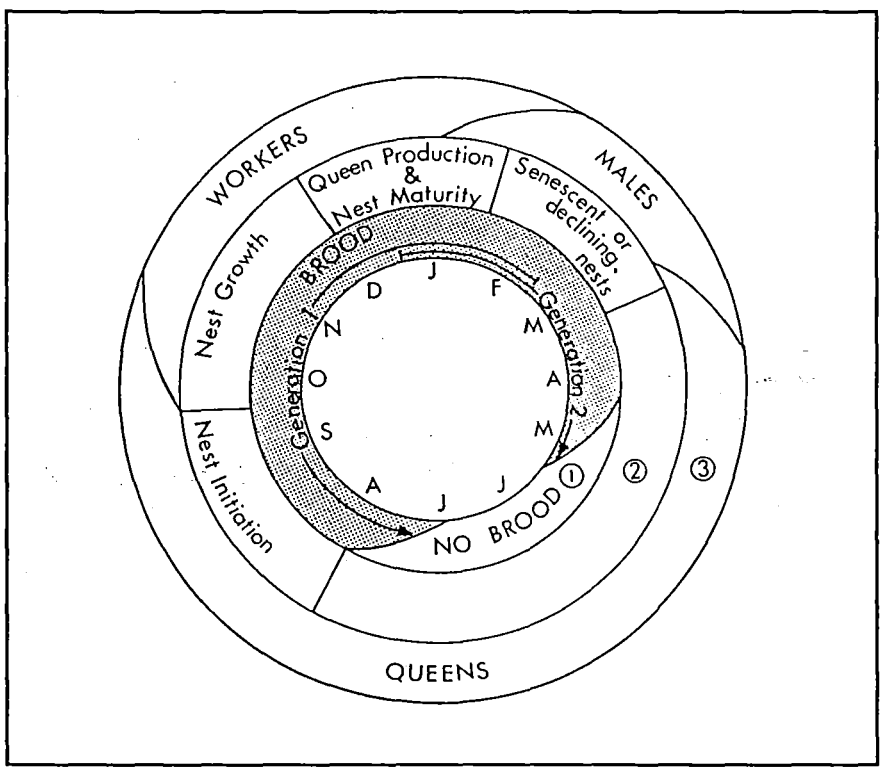


Figure 2.1. Generalised bumble bee life cycle in New Zealand: 1. Presence of brood. 2. Stage of nest development. 3. Periods when each caste is dominant on flowers. (source: Donovan & Macfarlane 1984).

Nesting, hibernation and forage requirements

For both hibernation and nesting, bumble bee queens require areas of undisturbed, well drained, warm, sheltered, dense vegetation. For forage, bumble bee workers generally prefer perennial flower species (Saville 1993; Osborne 1996). In agricultural landscapes where cultivation, clearing, and spraying of chemicals take place, nests sites are limited, as are forage resources which result in a dominance of poorly rewarding annual flower species (Corbet *et al.* 1994). Poor pollination rates by bumble bees are usually a result of overall bumble bee populations being too low. In Europe there has been a steady decline in native bumble bee species and abundance with intensified land-use, and patterns of habitat fragmentation (Williams 1982).

2.2.2 Bumble bee foraging biology and pollination

Bumble bees have particular morphological, physiological, and behavioural specialisations which contribute to their role as pollinators of several flower species, in environments where other pollinators are limited. Several foraging specialisations of bumble bees are reviewed in detail by Heinrich (1976). Key factors are summarised and described below.

Morphological and physiological specialisations of bumble bees

Because of their large, robust bodies, bumble bees are strong fliers in windy and wet conditions which limit other species, including honey bees (Donovan & Macfarlane 1984). Their large densely hairy bodies transfer large amounts of pollen, and increase the rate of pollen contact with flower stigmas (Donovan & Macfarlane 1984). Some specialised bumble bee species' tongue lengths match that of certain long corolla-tubed flowers, at the base of which the nectaries are positioned (Heinrich 1976). Differences in tongue length between bumble bee species and between other bee species enables the partitioning of resources to certain species, and hence, the avoidance of competition (Corbet 1995). For example, long-tongued bumble bees such as *B. hortorum* (L.) can reach the nectaries of tetraploid long corolla-tubed red clover varieties of *Trifolium pratense* (L.; Leguminosae), whereas certain short-tongued bumble bees cannot do this, at least "legally". Short-tongued bumble bees are opportunists, and can "rob" long-corolla-tubed flowers in some cases, by biting holes in the base of the flower to extract nectar without entering the flower. In this case, flowers are not always pollinated (Faegri & van der Pijl 1979). Bumble bees have a large honey crop and work faster than honey bees (Patten *et al.* 1993). Honey bees visit about 7-17 flowers per minute, while bumble bees can visit 10-30 in this time (Free 1993).

"Thermoregulation" is highly advantageous and bumble bees have developed this to enable flight in temperature conditions in which other bees can not fly (Heinrich 1979d). Bumble bees can both actively retain heat and perform active heat loss to keep their muscle temperature between 30° C and 40° C (Heinrich 1979d). Thermoregulation can be achieved through a biochemical process and through physical "shivering" (Prys-Jones & Corbet 1991). Bumble bees have been observed foraging at ambient temperatures of -3.6° C in Arctic conditions, and

in very hot desert conditions of 46° C (Barth 1991), the highest for any insect recorded. Bumble bees achieve this by using regurgitated nectar droplets held on the tongue, which, by evaporating can cause cooling of 10° C. Most solitary bees and honey bees cannot fly below 16° C. Honey bees, the main competitors of bumble bees, cannot fly at temperatures below 11° C or over 33° C (Wratt 1968).

Legume flowers which need to be "tripped" provide an example in which bumble bees are of significant value because of their morphology. The stamens and stigma of the lucerne flower are concealed within the flower. When an insect alights, a membrane splits and the staminal column springs back, knocks the bee on the head and releases pollen to be spread to the next flower's stigma that the insect visits. The tripping mechanism is avoided by several insect species including honey bees because it is physically-demanding. Because bumble bees are large and have strong exoskeletons they will readily visit legume flowers.

Behavioural specialisations of bumble bees; foraging strategies

Certain typical foraging habits or "strategies" have been recognised in bumble bee workers in relation to the spatial location of flower resources. Terminology encountered in the literature used to describe some of the key patterns established include "traplining" (Thomson *et al.* 1987), "majoring and minoring" (Heinrich 1976), "area-restricted searching behaviour" (see Thomson *et al.* 1982), and "patch loyalty " ('fidelity"). Each behaviour has characteristic distance and direction patterns associated, and some behaviours are closely related to each other.

Some authors describe, from a theoretical basis; "foraging rules" or foraging "decisions", which are "moves" a bumble bee should make if it were to maximise the rate of energy gain (i.e., collect the maximum amount of carbohydrate per unit time). This terminology is associated with studies using bees as models to test "optimal foraging theory" theories (see (Pyke 1984) for description and review. Mostly, these studies have been carried at a small scale (between flowers) and many in artificial laboratory conditions, which test predictions based on complex computer models. The detail of these are beyond the scope of this review.

"Traplining"; Darwin (1876) first described the predictable sequences or foraging routes ("traplines") of bumble bees. Thomson *et al.* (1982) described traplining behaviour between patches of *Aralia hispida* (Vent.; Araliaceae), and concluded that this strategy enabled time to be saved searching for new flowers to visit each bout.

"Majoring and minoring"; Bumble bees tend to concentrate their foraging efforts on one "major" flower species, while constantly sampling ("minoring") on a wider range of flower species (Heinrich 1976; Heinrich 1979b). This enables them to switch to a new "major" species when a more profitable source of nectar and pollen becomes available. Related to majoring and minoring behaviour is "flower constancy". This is the likelihood of bumble bees repeatedly to visit the same flower species (see Brian 1954b; Heinrich *et al.* 1977; Schneider & Glass 1990; Teräs 1981). The term "floral -visitation-sequences" was coined by Waddington (1983a) who considered it more accurately to describe the relative proportions of flower species visited during a bout than "flower constancy", which tends to assume bumble bees always major on one flower species.

"Area-restricted searching"; This behaviour is the tendency of a forager, once reaching a rich resource to "fix" itself and limit searching to a small area in an effort to encounter a more rich resource (Heinrich 1979e). Area-restricted searching behaviour is characterised by sharp turning angles and short flight distances to nearby flowers (Heinrich 1979a). "Nearest-neighbour" (i.e, moves to nearby flowers from the one just visited) visitation has been explained by the need of the bee to minimise flight distance and time in order to maximise pollen and nectar rewards. Short flight distances are characteristic of area-restricted searching behaviour predicted by Pyke (1978). Conversely, after an unrewarding flower or a sparse patch is encountered, bumble bees should exhibit straight paths with little angular deviation and increase flight distances to increase their chances of finding another rich patch (Heinrich 1979c). This pattern is comparable with patterns found in the predator-prey literature (Putman & Wratten 1984).

Literature reports are mixed regarding the existence of area-restricted foraging behaviour by bumble bees. Heinrich (1979a) found that on a plot of white clover which had previously been

covered to exclude other insects so nectar and pollen was abundant, bumble bees produced short flights with sharp angular movements rather than long flights and broad turning movements made between flower heads in depleted areas. Soltz (1986) also hypothesised that it would be advantageous for bumble bees to show this behaviour, but in her work did not confirm the existence of area-restricted behaviour. Zimmerman (1982) suggested that, rather than landing and searching, bumble bees can differentiate reward quality before landing, perhaps visually, which has implications for observed movement patterns.

"Patch loyalty" or " patch fidelity" refers to the tendency of bumble bees to return to the same patch, or same area within a patch to forage between bouts; bumble bees do exhibit area fidelity under some conditions (Waddington 1983b). In recent mark-reobservation experiments, for example, Saville *et al.* (1996) demonstrated considerable patch loyalty by bumble bees in a landscape-scale study in farmland area. Several bumble bees returned repeatedly to within 25 m of the point at which they were marked.

2.3 The importance of pollination to farmland flora

Bumble bees are particularly important pollen vectors for many entomophilous crops and wild flowers which require cross pollination, and can also improve success rates in partly self-fertile or wind pollinated species (Corbet *et al.* 1991). Bumble bees are of particular importance, or offer potential as key pollinators in cool climates. Both New Zealand and Norway experience temperate to cool climates. The following section gives background to the two farmland study sites involved in this thesis. One concerns pollination of legumes by bumble bees to improve seed yield in commercial crops in New Zealand, and the other involves the preservation of flower species of conservation value in traditional subsistence farm meadows in Norway.

2.3.1 Conservation areas in Europe; "The Cultural Landscape"

Unique Scandinavian "Cultural Landscapes" exist in parts of Sweden (Berglund 1991) and Norway (Birks *et al.* 1988) which are of enormous conservation and heritage value to Scandinavian people. Cultural landscapes are the result of several thousands of years of interactions between humans and nature (Birks *et al.* 1988). Some traditionally-managed landscapes date from at least the Iron Age (Norderhaug 1988). Typical Cultural Landscapes include the subsistence farms of western Norway. Because of changes in technology and life-styles over the centuries in northern Europe, many operational cultural landscapes have been lost, either through abandonment or modernisation. However some traditional landscapes lingered on in the mountainous regions of western Norway due to the rugged topography which has limited the access of high-technology (Birks *et al.* 1988). "The future of such landscapes is totally dependent on suitable agricultural management" (Fry 1991). A few remaining Cultural Landscapes are now the subject of Norwegian Government-led projects aimed at the restoration and preservation of these sites of significant heritage value. The Norwegian Institute for Nature Research (NINA) has responsibility for "The Cultural Landscape" programme, with which Chapter 3 of this thesis was involved.

Cultural landscapes include physical structures (such as log cabins and stone walls), and specific vegetation types created by man (see Norderhaug 1988). Traditional low input-low output systems over several centuries have resulted in a wide diversity of plant, animal and invertebrate species (Norderhaug 1988). "The old cultural landscapes of Scandinavia should be regarded as biological cultural monuments". They contain an ecological diversity as a result of long established farm methods, which is not present in the natural landscape and which is diminishing in the modern agrarian landscape" (Austad 1994).

Following abandonment, a form of habitat fragmentation is occurring. Meadow clearings are increasingly becoming smaller and more isolated from each other as the surrounding spruce and birch forests rapidly regenerate. There are concerns that this may have impacts on the biological diversity of herb-rich hay meadows (see Plate 2.1). Losvik (1988) suggested that habitat fragmentation effects are responsible for the loss of biological diversity in some Norwegian

meadows. In addition to scrub invasion, use of nitrogen fertilisers, urbanisation and planting of Spruce have contributed to the decline of herb-rich hay meadows (Norderhaug 1988). In Sweden, Ingelög (1988) documented declines in a range of species in association with changes in traditional agricultural landscape use. Høiland (1993) and Norderhaug (1993), both cited in Fry (1993b) report that there are indications that similar processes are occurring in Norway for both traditional arable weeds and meadow species. Fragmentation effects contributing to species declines have been reported for a wide range of species including invertebrates, birds, frogs and some plants (Jennersten *et al.* 1992; Opdam 1990). A key aim of the Norwegian Cultural Habitat programme is to evaluate effects of habitat fragmentation on biological diversity in Norwegian herb-rich hay meadows.

Pollination is a "keystone process" in maintaining biological diversity (Kevan 1991). Jennersten (1988) showed that in Sweden, habitat fragmentation had detrimental effects on the pollination and reproductive success (seed set) of a butterfly-pollinated species *Dianthus deltoides* (L.; Caryophyllaceae) because of limited pollinator service between plant populations on isolated habitat remnant "islands" which had become surrounded by intensively managed agricultural areas. There is also evidence from other parts of the world that fragmentation adversely affects pollinator efficacy (see Corbet *et al.* 1991; Laverty 1992; Rathcke & Jules 1993; Aizen & Feinsinger 1994; Buchmann & Nabhan 1996). Bumble bees appear to be useful bioindicators of habitat quality and land use in the Norwegian countryside. Strong correlations have been demonstrated between the number of bumble bee species and the number of species of flowers (Fry pers. comm.; see Osborne 1996). This suggests that they play a key role in the maintenance of plant biological diversity. Limited movement by pollinators, particularly by native bumble bees within and between Norwegian meadows is therefore possibly a major contributing factor to the current decline of flowering-plant species diversity and abundance, as a result of limited pollen flow within and between plant populations (Fry 1993b).

It is hoped that in the future information on the effects of habitat isolation on bumble bee foraging behaviour can be combined with data on the reproductive success of bumblebee-pollinated flora in the meadows.

Plate 2.1. Elements of a traditional cultural landscape in a herb-rich meadow in Hjartdal, Southern Norway, highlighting a wide diversity of flowering plant species in which traditional management techniques are still operational. (Photo A. Norderhaug).

2.3.2 Commercial crops in New Zealand; Legume seed crops

New Zealand's land-use pattern contrasts markedly with that of Norway's. New Zealand has extensive pastoral and cropping farmland. Much of New Zealand's agricultural production is based on pastoral systems in which legumes are a vital component. New Zealand is famous for the first successful introduction of an insect species expressly for pollination (Donovan 1990). Soon after Darwin showed that red clover depended upon bumble bees for pollination, they were introduced into New Zealand, and red clover yields dramatically increased as bumble bees became relatively common (Hopkins 1914 cited in Donovan 1990). Lucerne seed production however provides a "vexed problem" (Doull 1967). Poor pollination rates have been identified as a contributory factor to the sub-optimal yields currently achieved (Dunbier *et al.* 1983).

Poor pollination rates: a key limitation to lucerne seed production

In New Zealand, important legumes grown for seed include red clover *Trifolium pratense* , white clover *Trifolium repens* (L.; Leguminosae) and lucerne *Medicago sativa.* (Webb *et al.* 1988). Clover seed exports earn millions of dollars each year. Lucerne yields however are very inconsistent between locations and years (Doull 1967). Some high yields have been achieved (>1000 kg/ha) but are mostly low and unprofitable (< 200 kg/ha) and in some years supplementary seed must be imported. In 1983, New Zealand imported 70% of its lucerne seed from the US. (Dunbier *et al.* 1983). This figure has probably decreased due to a more specialist approach taken by growers since the 1980's but a significant amount is still imported (Purves pers. comm.).

Low yields have been linked to pollination shortfalls (Doull 1967; Dunbier *et al.* 1983). New Zealand's relatively cool climate for producing lucerne seed is often described as marginal for plant development and bee activity, while the bee fauna itself is poor compared to high yielding overseas crops in the hot semi-arid regions of Western America, Israel and parts of Australia where consistent yields of 1000 kg/ha or higher are achieved (Donovan 1974). Honey bees, regarded as the major lucerne pollinators in many hotter overseas climates in which tripping is easier are not as effective in New Zealand's cooler climate (Free 1993). In New Zealand, honey bees act mostly as nectar robbers, being light enough to enter the flower sideways, avoiding the tripping mechanism and therefore avoiding flower pollination (Donovan & Macfarlane 1984). Management techniques such as scent training, swamping, and re-introduction of naive bees which attempt to force honey bees to visit crops can be costly and not guarantee success (Jay 1986). Improving pollination rates should be regarded as part of a specialist seed production approach to reduce New Zealand's large dependence on imported seed (Dunbier *et al.* 1983).

The potential of bumble bees to increase lucerne seed yields

Bumble bees have been identified as good pollinators of lucerne in New Zealand (Read *et al.* 1989; Gurr 1955; Gurr 1974). High bumble bee populations have been associated with very high yields of lucerne in certain localities of New Zealand (Gurr 1957). Their ability to thermoregulate allows them to be active when low temperatures limit the activity of honey bees, lucerne leafcutting bees *Megachile rotundata* (F.) (Megachilidae) and alkali bees *Nomia*

melanderi Cockerell (Halictidae). Historical meteorological records show that conditions for non-bumble bee species are frequently not met in Canterbury. Summer conditions are associated with predominating strong northerly winds (Cherry 1994) and minimum daily temperature requirements for the flight activity of introduced solitary bees (16° C with sunny conditions) are frequently not met. During the peak flowering period of many legumes, flight of solitary bees is limited, because of the cooling effects of these winds, and because they are not robust fliers in windy conditions (Purves pers. comm.).

Limitations and potential of bumble bees as field crop pollinators

Bumble bee populations fluctuate greatly between localities and seasons and usually are too low. If yields are to be improved through bumble bee pollination, bumble bee behaviours need to be understood in order to enhance their populations and effectiveness in seed growing areas to a consistent level. In recent years, bumble bee-rearing technology and efficiency have vastly improved. New Zealand companies export colonies overseas and also supply them to the local market, mainly for pollination of glasshouse crops. Until recently, the use of commercially reared nests was not viable for outdoor crops (Pomeroy 1988; Donovan 1990). Now large and efficient rearing operations allow the production of quality colonies year-round. Perhaps now the use of them in high value outdoor crops is a viable cost option. There is an increasing number of high value outdoor crops being produced in the absence of their original pollinators for which locally introduced or enhanced populations of bumble bees may be beneficial. In New Zealand these include kiwifruit, novel herb crops, melons, cranberries and legume seed crops. Field-colonised *B. hortorum* nests are currently used by red clover seed growers in Canterbury (Donovan pers. comm.). Key advantages are that bumble bee nests are small and easy to move, and being annual, require minimal input once placed in the field.

Contribution of spatial data in improving management of bumble bees

Historically, studies in New Zealand have focused on the temporal aspects of bumble bee foraging. Various predictive bee stocking rate models have been based on this data (e.g., Macfarlane & van den Ende 1989). Spatial information will be a beneficial addition to this temporal knowledge. Examples of bumble bee management trials in New Zealand which could benefit from movement information include: 1. the arrangement of permanent nest hives in

"pollinator refuges" (see Pomeroy 1981; Donovan & Wier 1978), 2. the placement of field-occupied hives to new crop sites (see Read *et al.* 1989), 3. for the placement of commercially reared nests into high value outdoor field crops (see Pomeroy 1988; Macfarlane *et al.* 1994) and 4. in the enhancement of local bumble bee populations (see Clifford 1973). Renewed interest in developing forage and nesting site refuges to increase bumble bee field populations is in progress at Lincoln University (Barron pers. comm.). Since 1980, 12 to 30 nests annually of *B. hortorum* have been used for pollination of red clover. In 1985, 12 nests of *B. terrestris* (L.) were used for pollination of kiwifruit (*Actinidia delicosa* Chev.) and lucerne *Medicago sativa* (Donovan 1990).

If it is known what area a nest of bees can service, and what factors affect it, nests can be placed accordingly to the estimated bee stocking rate and in the most effective locations. Accurate movement information is essential for setting isolation distances between pure seed crops to avoid genetic contamination through cross-pollination (George 1985).

2.4 Previous insect movement studies

Different types of insect movement include migration, mating, foraging, defence and swarming. Important parameters of movement include distance, height, direction and speed. Knowledge of movement at various scales is important for optimal management of pest, beneficial and other insects which may be of medical, economic and conservation importance. Pollination is an important example in which insect movement has benefits for both economic and conservation goals.

Pest and disease eradication programmes can be planned more efficiently if the range of the pest is known. In Israel, knowledge of movement distances for the malaria transmitting *Anopheles* spp. mosquitoes has allowed the recent development of efficient computerised surveillance systems, so that if an outbreak occurs, prompt control measures can target the likely breeding site, a specific *Anopheles* vector and a probable human source (Kitron *et al.* 1994). The establishment of quarantine zones around areas of a recent direct economic pest (Mediterranean

fruit-fly *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) outbreak in New Zealand highlights the application of movement knowledge in control programmes. Monitoring, and predicting the spread of undesirable species such as Africanised bees requires the constant accumulation of movement information (Otis *et al.* 1981). The foraging range of honey bee vectors will influence the spread of fireblight pathogen agent in orchards (Corliss & Adams 1992). Bees may also be responsible for the spread of sexually transmitted plant diseases such as *Ustilago* (Jennersten & Kwak 1991).

The success of biological control programmes often depends on the mobility and adaptability of the agent (Stiling 1983). Mauremootoo *et al.* (1995) showed that hedgerows slowed movement of carabid beetles compared with open barley field controls. Evans (1996) reported that hedges were significant impediments to hover fly movement.

Knowledge of movements of insects of conservation value enables a better understanding of their habitat usage. This is important in the development of management programmes. For example, butterfly movement can be significantly impeded by hedgerows in the landscape acting as barriers (Fry & Robson 1994).

2.4.1 Movement studies of foraging bees (Hymenoptera)

Because of their world-wide value as domesticated crop pollinators, most movement studies involve honey bees (see Free 1993). Conservation-related studies which are less common usually involve native bee species which have coevolved with native flora (e.g., Kwak *et al.* 1991), rather than honey bees which have been relatively recent introductions in many countries. The conservation of bee species themselves is an added impetus to study patterns of habitat usage and requirements (Day 1991; Williams 1996). Bees also provide ideal subjects for testing optimal foraging theories (see review by Pyke *et al.* 1977), communication theories such as the famous "honey bee dance" (von Frisch 1967; Lindauer 1971), and to provide evolutionary viewpoints on the development of social behaviour which may be related to other organisms and coevolution with plants (Michener 1974).

Optimal foraging theory- a theoretical basis for predicting pollinator movement patterns

"Optimal foraging theory" is a theoretical framework developed to help categorise and explain foraging behaviour. It has provided a major basis for pollinator movement studies. It involves four main issues: 1. Where to forage, 2. What to eat, 3. What patch to take and how long to stay Pyke *et al.* (1977). The goal of many foraging strategies is thought to be maximisation of energy gain (i.e., maximum amount of carbohydrate collected) per unit effort or time (Soltz 1986). Bumble bees foraging for nectar have been considered ideal subjects for testing optimal foraging theories because nectar resources are easy to manipulate, there appears to be little predation on and few interactions between foraging workers (Soltz 1986), and bumble bees are "central place foragers" (Plowright & Laverty 1984). Several studies of bumble bee movement have stemmed from studies testing optimal foraging theory.

2.4.2 The spatial approach and a review of bumble bee movement studies

As outlined in Section 2, bumble bees do not communicate on the same level as honey bees. In addition, there are several morphological, physiological and behavioural differences between the *Apis* and *Bombus* genera indicating that the same movement patterns of honey bees should not necessarily be applied to bumble bees. Similarly, findings in the few studies of movement of solitary bees are not relevant to bumble bees.

This section firstly outlines the parameters important to movement studies: distance, direction, and foraging area. The theme of "scales" of study is then introduced, and discussed in relation to key findings of bumble bee movement studies relevant to this thesis.

A spatial approach

In evaluating and predicting the efficiency of bee movement, temporal aspects of foraging should be considered in conjunction with, not in isolation from, spatial aspects of foraging. For example, a bee may forage at a fast rate, but if foraging is confined to a small area within a patch of flowers to one patch in a whole mosaic of patches, cross-pollination services may be limited between flowers on different plants and between different populations. In addition, the

efficiency of bumble bees as a colony unit should be considered, rather than focusing on the behaviour of individuals as isolated studies.

Key spatial parameters: flight distance, flight direction and foraging area

The two common parameters used to quantify movement patterns are distance (linear distance between two flowers or patches) and direction (commonly measured by differences in angular directions and defined in degrees) (Kearns & Inouye 1993; Waddington 1979). Together these measurements can estimate foraging areas. This thesis is concerned with the spatial distribution of foraging bumble bees only. c.f. the "dispersal" of reproductives or non-foraging activities.

Flight distance

Flight distance, measured as the linear distance between two foraging points of a pollinator Both have implications for the success of pollen transport and hence cross-pollination between flowers, patches, and whole populations. Authors which have specifically considered flight distances include Waser 1982; Pyke 1979; Ott *et al.* 1985.

Distance studies on a large scale can be biased because long-distance flights can not always be recorded (Kearns & Inouye 1993). Leptokurtic distribution curves of flight distances of many species, including bumble bees are prevalent in the literature. It is likely that in large scale studies, where the whole area can not physically be reobserved, that these are artefacts of sampling method (Kearns & Inouye 1993). The linear "transect method" in which individuals are recorded along a standard walk is a common method used for reobservations of marked bumble bees (see Dramstad 1996a). As linear distance increases from the marking point, the area of the circle (used to estimate foraging area from the colony) based on the radius of that length, increases disproportionately to the radius length. This means that the probability of reobservation with increasing distance also decreases disproportionately. The resulting leptokurtic curve therefore, peaks at short flight distances, then steeply trails off with increasing flight distance. Ideally a mathematical correction should be made to account for this quandary (Fry pers. comm.).

Flight direction

If a pollinator is foraging optimally one would expect it to move directionally in order to avoid re-visiting just-emptied flowers (Pyke & Cartar 1992). Directional movement by the pollinator would also have advantages for the outcrossing of pollen between plants and between plant populations, reducing selfing and avoiding inbreeding depression (Handel 1983).

Directionality has been classified in two main ways; "Overall directionality" (the tendency for the majority of flights to be in one direction or within the same 180° category (as per Woodell 1978) and "sequential directionality" (the tendency of a flight to follow the direction of a previous flight (e.g., Pyke 1978). Measuring directionality by angles of departure relative to angles of arrival to each new point (usually to a new flower) is the most common method used in the literature (e.g., Levin *et al.* 1971; Pyke 1978; Zimmerman 1979; Waddington 1979). The "angle of departure" can range between -180° to +180°, with a straight ahead move equal to 0°. If the frequency distribution drawn from all collated moves in the foraging bout is unimodal with a mean of approximately 0°, strong directionality is exhibited by the forager (i.e., most moves are straight ahead). Random movement is reflected by a flat distribution (Zimmerman 1979). However, Kipp (1989) cautioned that relative angular measurements can produce results which are fraught with computational artefacts, are difficult to interpret, and can be misleading in their biological interpretation. Woodell's (1978) method was more simple, in which overall directionality was measured, based on compass directions relative to the observer rather than relative to the bee's arrival point.

There is debate between authors about the mechanisms controlling directionality. Ginsberg (1986) for example, concluded that honey bee directionality is largely controlled by external cues and is not completely an artefact of resource distribution, and this could be widespread in the Hymenoptera including bumble bees. Pyke & Cartar (1992) and Kipp *et al.* (1989) found evidence for innate-based foraging decisions in bumble bees. Pyke & Cartar (1992) proposed that directionality may operate because a bumble bee prefers to leave a patch in the same direction as it approached it, the "arrival hypothesis", or left it; the "last-faced hypothesis".

In contrast, others argue that directionality is governed by flower distribution, including plant and flower architecture (see Heinrich 1979e; Zimmerman 1979; Kipp *et al.* 1989; Rasmussen & Brodsgaard 1992). Authors who suggest differences are due to the spatio-temporal location of pollen and nectar rewards include Pyke (1978), Heinrich (1979c) and Morse (1980). Directionality in bumble bees was correlated with abiotic microclimate effects by Woodell (1978) who concluded that bumble bees were directional in wind because they could follow odour streams and land easier on flowers upwind. Bumble bees clearly reversed their flight direction in response to reversed wind direction. Pasek (1988) also reported that directionality patterns of insect movement were affected by wind and windbreaks.

Foraging areas

Foraging area has been used to refer to both individual bumble bees' use of space once they are in a patch (i.e., "forage patch"), or, to describe the whole "activity zone" of the colony. Terminology to describe foraging bumble bees' use of space is inconsistent and often confused (see review in Dramstad 1996a). Because bumble bees are "central place foragers" (see Covich 1976), it is appropriate to quantify foraging areas in terms of a circle's area derived from a known radius from the nest where the bee or bees are observed (Heinrich 1979a).

Within patch foraging areas are often quantified by polygonal area arbitrarily drawn around the outermost points of the bees' foraging points (measured in m^2) (e.g., Heinrich 1976), or simple squares or rectangles as done by Singh (1948) for honey bees. Heinrich (1976) found that bumble bees foraged in very limited specific areas of 7 m^2 in a meadow compared with some very large, or unmeasurable areas in a large hayfield. Waddington (1983b) pointed out that bumble bees are more likely to exhibit area-fidelity in environments with distinctive landmarks and topography. Handel (1983) also reported that some bumble bees, like honey bees, tend to forage within sharply delineated areas.

A spatial hierarchy of "scales"

A recent trend in approaching and organising movement studies of different organisms by landscape ecologists has been to recognise a spatial hierarchy of "scales" (Forman & Godron 1986). "Scale" is "the level of spatial resolution perceived or considered" , and which scale a

study is based on depends on the animal and questions involved. The aim of landscape ecologists is to link patterns and processes, some of which, in mosaic patterns in heterogeneous landscapes, are only clear at certain scales (Forman & Godron 1986).

Pollination is a "keystone-process" in several landscapes (Kevan 1991) influencing the spatial patterns of resource distribution observed. Pollen flow through pollination services may have different implications at different scales. For pollination studies this can be translated to a scale structure ranging from large landscape scale (between large areas- fields for example) to small between-flower studies. This framework can allow comparisons between related studies (e.g., plant genetic studies). Resolution of measurement scale will depend on the plant species or habitat involved, but in this thesis, study scales are categorised into: "small scale" movements between flowers (cm and m), "medium scale" movements between patches (up to several hundred metres apart) and "large scale" or "landscape level" studies over and between large areas of non-uniform habitat (several hectares).

Bumble bee-movement studies at a small scale

The majority of bumble bee movement studies have been carried out at the "small scale" between flowers and within patches. Several studies have involved testing aspects of optimal foraging theories measuring distance and directionality in relation to nectar and pollen energy "profits" (see review in Waddington 1983b). Models have been developed to describe and predict movements within inflorescences (e.g., Best & Bierzychudek 1982) and among inflorescences (Kearns & Inouye 1993), and many authors have used "artificial-flower patches" (e.g., (Hartling & Plowright 1979), in which resources can be manipulated.

Directionality is the key parameter which has been assessed in small scale studies. A large number of small-scale bumble bee movement studies have been carried out on vertical spike-flowers. Bumble bees exhibit consistent patterns of directionality and distances moved on protandrous infloresences arranged in spikes. They show marked directionality between inflorescences on species such as monkshood *Aconitum septentrionale* (Koelle; Ranunculaceae) (Prys-Jones & Corbet 1991), *Delphinium* species (Pyke 1978), rosebay willowherb *Epilobium angustifolium* L. (Benham 1970) and foxglove *Digitalis purpurea* L.

(Best & Bierzychudeck 1982). Individuals tend to begin foraging at the bottom of the inflorescence, then move methodically up and around the spike directionally to avoid revisitation of just-emptied flowers. Generally moves are to the nearest neighbour flowers. Spikes are "protandrous", i.e., each flower is male when it first opens (offering stamens only) and tends to be at the top of the inflorescence, and female ones, which are older and offer stigmas later, are at the bottom of the spike. As bumble bees travel up the spike, they pick up the male pollen last, which is then deposited on the stigma of the next inflorescence when they begin to forage at the bottom (female) flowers again, hence effecting cross- pollination (Heinrich 1979a). Plowright & Laverty (1984) suggested that these patterns may be an evolved behaviour in relation to the predictable sequence of rewards found on vertical inflorescences.

In contrast to movement patterns on vertical spikes, patterns within horizontally-arranged swards are poorly understood. A pattern which is common however, is the tendency for bumble bees to move to nearest neighbour flowers (Waddington 1981). Nearest-neighbour visitation has been explained by the need of the bee to minimise flight distance and time in order to maximise profits. Short flight distances are characteristic of the area-restricted searching behaviour predicted by Pyke (1978).

Most models (based on optimal foraging theory goals in which the forager strives to maximise carbohydrate intake) predict that it is inefficient for a forager to recross its own path, and can be avoided by alternating left and right turns (Zimmerman 1979). Similar patterns have been observed in butterflies, birds, fish and bees (Zimmerman 1979). Several studies of foraging by flower visitors demonstrate that distance and direction behaviour changes in response to changes in nectar or pollen reward and to spatial arrangement of flowers (Kearns & Inouye 1993), apparently in ways which maximise net energy gain by foragers (e.g., Pyke 1978). For example, Heinrich (1979c) found that on a white clover patch which had been caged to exclude other insects from taking nectar and pollen, bumble bees displayed random foraging patterns. Once they reached the "rich" area, they rapidly increased their rate of turning and turned sharper angles, and increased the number of short flights made to "nearest neighbour" flowers, significantly restricting their search area. Bumble bees did not persist in moving straight ahead if rewards were good. However, results from different experiments have shown bumble bee

patterns to be highly variable, and, as outlined, interpretations to explain either directional or random behaviours are mixed between external environmental cues and internal (innate behavioural) mechanisms.

Medium and large scale movement

Studies between distinct patches are more limited. In one such study, Rasmussen & Brodsgaard (1992) showed that in populations of Birdsfoot-trefoil *Lotus corniculatus* L. bees usually visited nearest-neighbour plants, showed no marked directionality, and foraged mostly within patches. About 3% of flights recorded were between patches and considerable pollen carry-over resulted. Rasmussen's work highlights the need to combine spatial patterns of pollinator movement with plant genetic data in order to evaluate the efficacy of pollinators. It is possible that only a small number of inter-patch visits are sufficient to maintain genetic stability in isolated plant populations. Saville *et al.* (1996) reported that bumble bees showed considerable patch loyalty to forage areas, in which they were marked, but were also recorded up to 300 m between forage patches.

Large scale studies are the most limited in the literature. This shortage is probably because of the difficulties involved in organising and carrying out work in the field at a large scale. Recently more authors have addressed aspects of large scale bumble bee movement in modern fragmented agricultural habitats (see Banaszak 1983; Dramstad 1996a; Saville 1996). Bowers (1985) in the US and Kwak *et al.* (1989) in The Netherlands have examined aspects of bumble bee movement in more natural habitats. There are several anecdotal reports of bumble bee foraging distances at large scales, and predictions that bumble bees will probably travel at least 5 km if necessary (Heinrich 1979a). Findings relevant to the work in this thesis will now be discussed in terms of movement from the nest, and movement in relation to crops.

Bumble bee movement from the nest and in relation to crops

There is some data for honey bee movement from their nest but very little is known about bumble bees, and even less about solitary bees and movement of other pollinator groups such as Diptera (Kearns & Inouye 1993). For some bee species, e.g., leafcutting bee *Megachile rotundata* foraging areas are very clearly restricted. Aerial photographs of alsike clover

Trifolium hybridum L. crops in Canada showed distinct "seed rings" (brown patches of dying-off pollinated flowers) 30 m in diameter around each leafcutter bee shelter (Fairey & Lefkovitch 1993). However, such patterns are not as distinct for bumble bees. There have been few specific studies of bumble bee dispersal from the nest (Kearns & Inouye 1993).

It is generally assumed that bumble bees forage within a few hundred metres of their nest (see review in Dramstad 1996b). Most reports of bumble bee movement in relation to the nest are anecdotal, with experiments not specifically designed to address foraging area position questions. Dramstad (1996b) questioned the statistical evidence for these assumptions, and in a series of experiments involving reobservations of nest marked bees, concluded that bumble bees may forage much further than has been assumed- over several hundreds of metres, often being "missing" from the study area, because their large flight distances had not been allowed for in trial design. She suggested that bumble bees may actually "prefer" to forage far from their nest, as an evolved behaviour to avoid predation.

In comparison, Heinrich (1976) argued that "as long as food is locally abundant, it is probable that most bumble bees forage close to the nest". Macfarlane *et al.* (1994) and Crane (1990) reported commercial colonies to have less than one third the range of feral bumble bee colonies. Macfarlane *et al.* (1994) cited the usual foraging range of bumble bees as from 200 to 600 m, which was also supported by Patten *et al.* (1993). Free & Butler (1959) marked bumble bees from an artificially reared nest placed in a 1.24 red clover crop, and mainly relocated bees close to the nest. Braun *et al.* (1956) cited in Saville *et al.* (1996) placed nests near large 30 and 90 ha red clover fields (presumably around the outside). No bees were found in the centre, a result which was attributed to their foraging close to their nests.

Conclusions and assumptions concerning bumble bee movement in relation to the nest are often based on observations of just a few or only one individual. Butler (1951) for example claimed that marked *B. agrorum* (Fabr.) from marked nests placed in a red clover field were never seen more than 18.3 m from their nest , but no information is given on methodologies. Although this may be accurate for one individual, results can not be extrapolated to estimate where most bees forage most of the time.

Baker (1992) and Hågstrom (1992) cited in Saville (1993) moved garden-occupied bumble bee boxes of *B. pascuorum* (Scopoli) to a large patch of *Echium vulgare* L. and marked over 100 bees inside. Few marked bees were found more than 10 m from the nest, and none further than 30 m. Saville (1993) concluded that at least " in the presence of such an abundance of suitable forage close to the nest it seems that some bees do forage at short distances from the nest". In comparison Baker (1992) and Hågstrom (1992) cited in Saville 1993) also relocated boxes of *B. lucorum* (L.) in more heterogeneous areas with patches of *E. vulgare* , *Rubus fruticosis sensu lato* and *Sedum acre* L. Sixty marked bees were monitored in a dawn to dusk study, but only four bees were recovered within 200 m. In other experiments involving *B. pratorum* (L.), *B. hortorum* , *B. ruderarius* (Müller) and *B. pascuorum* by Saville (1993) very low reobservation rates were recorded in nest experiments. The same phenomenon of "mysteriously disappearing bees" was found in a series of experiments by Dramstad (1996a), also in rather heterogeneous environments.

Hobbs *et al.* (1961) placed artificial colonies of *B. terrestris* and *B. borealis* (Kirby) next to plots of clover where there were also natural colonies. Workers from natural nests visited clover plots, but ones from artificial colonies did not, even though there was a supposedly rich food source only metres away. In an experiment by Dramstad (1996a), workers from *B. lucorum/terrestris* nests increased their usage of a large area of *Phacelia tanacetifolia* (Benth.; Hydrophyllaceae) after nests had been moved 200 to 300 metres from the strip, compared with when the nests were adjacent to the strip, and argue that the belief that bumble bees prefer to forage as close to their nests commonly assumed in the literature, has no empirical basis.

Macfarlane *et al.* (1994) used reared nests placed in the field to service cranberry flowers in a cranberry-bog. They concluded that foraging decreased 140-160 m from the hive, and that the foraging area was mainly limited to 30 to 60 m from the hive. On this data they based stocking rate suggestions (4.5 to 10 colonies per hectare). However, there was no reobservation data presented to indicate how representative their reobservations were of the whole colony, it was not known whether the bees were from the introduced nests, or were present already, and distance measurements were likely to be an artefact of the sampling technique used, resulting in a leptokurtic distribution of flight distances (see Section 2.4.2). Macfarlane *et al.* (1990)

recommended at least six colonies per hectare to pollinate red clover. They remarked that with the "limited foraging range" of bumble bees, proper colony placement is critical. However, this is not clearly described and there is no spatial approach to describing bumble bee behaviour apart from density counts per hectare, where it is not clear whether bees were from feral or introduced colonies.

Several authors have assumed that bumble bees will forage as close to the nest as possible to maximise energy returns, because flight, and flight time itself is energetically costly. Individual bumble bee flights up to 1.2 km by *B. pennsylvanicus* de Geer were recorded in homing experiments by Rau (1924). Free & Butler (1959) found that very few bumble bees returned to the nest when released further than 1.7 km away.

Methods used to measure bumble bee movement

A thorough review and details of the range of methods used to establish foraging patterns of bees is given in Kearns & Inouye (1993). Approaches taken can be divided into two broad categories: 1. Following the individual and later transcribing flight paths for analysis (e.g., Woodell 1978; Thomson *et al.* 1987; Turchin *et al.* (1991), either mapping the flowers individually as Olesen & Warncke 1989 did or working within a grid for reference points as Hodges & Miller (1981) and Soltz (1986) did, or 2. Mark- reobservation studies which are more practical at a larger scale (see Dramstad 1996a; Saville 1993).

Common marking techniques include the use of numbered tags or coloured paint spots adhered to the top of the bee's thorax. A popular alternative method to trace bee movement employs radioactive substances dusted on flowers which bees transfer to other flowers they may visit. A Geiger counter can then detect which flowers bees have visited e.g., Lecomte & Pouvreau (1968). Fluorescent dusts have also been used to imitate pollen flow (e.g., Kwak 1979), with the advantage that flowers can be later scrutinised under an ultra-violet light source. A novel approach to gain knowledge on different bumble bee nests which may service the same fields, was to attempt to identify different nests' individuals through genetic techniques (see Estoup *et al.* 1995; Kennet 1995). A recent development which offers potential for pollinator movement studies involves a small radar "harmonic generating tag" system (Hornsby 1996).

2.5 The influence of plant features on bumble bee movement

Factors influencing bee movement through the insect's foraging decisions can be grouped into three main categories: bee-, environmental- and plant-related cues. Plant cues operate through plant morphology, temporal and spatial arrangement, chemical and visual signals, and through food rewards. Pollen and nectar are a major determinant of foraging "decisions" (see Section 2.2.2) in bumble bees (Heinrich 1979a; Morse 1980; Baker & Baker 1983b), and therefore influence their movement patterns.

For worker bumble bees, the energy-rich sugars in nectar are important for flight, foraging, storing honey supplies, and regurgitating to young brood. Pollen is the principal source of non-liquid food. It contains most of the essential nutrients and is rich in proteins and lipids to nourish new reproductive bees and young worker larvae (Stanley 1974). Food availability is the major determinant of bee colony growth and reproductive success (Woodward 1990).

Bumble bees are behaviourally flexible in changing their foraging patterns to concentrate on rich nectar food sources, as are honey bees (Morse 1980; Seeley *et al.* 1991). Responses to changes in pollen availability were demonstrated by Harder (1990) who showed that on flowers with better pollen reward, there were longer visits to flowers, visits to more flowers within an inflorescence, an increased frequency of revisits and an increased likelihood of grooming while flying between flowers which resulted in longer flights.

Components of nectar and pollen "attractive" to bees

It has been established that nectar volume, sugar concentration in nectar, and the amount of pollen offered by flowers influences bumble bee forging patterns. In discrimination based on nectar rewards, lepidopteran and large bee species tend to favour flowers, the nectar of which contains predominantly sucrose rather than other sugar types (Baker & Baker 1983b) and which are in the 20-50% concentration range (Eickwort & Ginsberg 1980). However, it is not well understood if there are other specific components of pollen and nectar which influence bumble bee foraging "decisions". It has been suggested that nitrogen content of pollen for example could be a determining factor (Stanley & Linskens 1974). The physical surface

structure of pollen grains is thought to affect digestibility (see Brown *et al.* 1992). Odour cues may also play a role in both nectar and pollen-type recognition (Plowright & Laverty 1984). It is beyond the scope of this review, and of this thesis, to explore this level of detail. For thorough reviews concerning the chemical composition of nectar see Cruden & Hermann (1983) and Baker & Baker (1983a). For pollen chemistry detailed analyses are provided by Stanley & Linskens (1974).

2.6 Summary

In summary bumble bees have been identified as efficient pollinators of both wild flowers and crops. Two farmland habitats have been described in which pollination by bumble bees is thought to be limited, but where there is potential to improve pollination rates by bumble bees. Studies so far on bumble bees have focused on temporal aspects of foraging behaviour. In both cases, an understanding of spatial knowledge will be beneficial. Most publications to date have concerned small scales (between flowers and within patches). There is a shortage of bumble bee movement studies at the landscape scale. Focused studies on dispersal of bumble bees from their nests were non-existent until recently. Dispersal patterns of bumble bees from their nest have implications for conservation and commercial management of natural or laboratory reared nests or field occupied domiciles. This thesis addresses bumble bee movement at all scales, but focuses on bumble bee movement at large scales, and in relation to the nest.

Chapter 3

Bombus spp. movement in a semi-natural habitat

3.1 Introduction

Studies presented in this chapter are the result of a pilot project, organised under the auspices of the Norwegian Cultural Landscape programme (see Chapter 2.3.1). One aim of the programme is to preserve the exceptional plant diversity which occurred as a result of traditional management techniques in meadow-farm systems in parts of Scandinavia. After abandonment in recent decades, meadow clearings have become increasingly isolated remnants as surrounding forests regenerate. With the landscape fragmentation process and changes in certain management practices (or in the absence of management), a decline in plant species diversity and abundance has become apparent, both in agricultural and semi-natural habitats in Norway and Sweden (Losvik 1988; Norderhaug 1988; Austad 1994). The full significance of fragmentation and isolation experienced by Norwegian meadow vegetation has yet to be documented (Framstad 1993). There is evidence from Sweden (Jennersten 1988) and other parts of the world that fragmentation adversely affects pollinator efficacy (Laverty 1992; Rathcke & Jules 1993; Aizen & Feinsinger 1994; Buchmann & Nabhan 1996). Limited movement by pollinators, particularly by native bumble bees within and between Norwegian meadows is possibly a contributing factor to the current decline of flowering-plant species diversity and abundance, as a result of limited pollen flow within and between plant populations (Fry 1993b).

The meadow site used in this thesis enabled the study of bumble bee movement on a larger scale, in a more structurally heterogeneous landscape with a wider diversity of flora than in the New Zealand crop study (Chapter 4). Results will provide field data needed to test computer-model predictions of insect movement currently being developed. Movement data would be valuable combined with temporal foraging data and plant-genetic data in predicting the likely persistence of plant biological diversity in these meadows under the present system, or under management intervention.

In this chapter, movement by bumble bees is assessed in a typical, abandoned Norwegian meadow system to assess their pollination potential. Section 3.3 deals with small scale movement between flowers and over a patch. Section 3.4 investigates large scale movement between patches and between meadows.

Pollinator flight distance and directionality have direct implications for the success of pollen flow, and are the two most commonly measured parameters of pollinator flight patterns (Kearns & Inouye 1993). Section 3.3 involves the analysis of directional data *per se*, and Section 3.4 focusses on distance data. Results are considered in relation to the location of major flower resources, landscape features and recognised behavioural strategies sometimes exhibited by bumble bees, which were outlined in Section 2.2.2

Specific objectives within this chapter were :

- to test directionality by bumble bees between flowers and over a whole patch
- to assess bumble bee mobility between distinct patches, to test whether bumble bees crossed between meadows, and to assess area "loyalty" by bumble bees.

3.2 Site description

Experiments were carried out in south-west Norway, Telemark county, Hjartdal area (59° 37'N, 8° 41'E) in July and August 1993. Three abandoned farm-meadows ("Svervelli", "Barn" and "Scout") within a forest mosaic comprised the study site of approximately 5 ha., ca. 600 m -700 m a.s.l. Meadow areas ranged from ca. 0.15 ha to 1.5 ha (Figure 3.1). Norway spruce *Picea abies* (L.) (Pinaceae) and birch *Betula* spp. (Betulaceae) dominated forest stands. Stock grazing had been absent for several years, allowing extensive birch regeneration on forest edges (Plates 3.1 and 3.2). A sketch map of the field site (Figure 3.1) was manually produced, based on aerial photographs taken in 1976, topographical maps, and was updated with current field measurements accounting for forest spread since 1976. Supplementary field-tape and compass direction data were based on coordinates of remaining forestry-mapping markers. This data was needed to orientate the map northward and to place accurately landscape features to scale within

it. The permanent field pegs correlated with topographical maps and aerial photograph coordinates. Where forest and meadow edges were not clearly defined because of rock faces, steep banks, clear-felled areas, paths and gaps, arbitrary boundaries were assigned. In particular, meadow edges at coordinates I3 to K3 (Figure 3.1) were not well defined. Transect lines, established for reobservation of marked bumble bees (see Section 3.4.2), were extended significantly beyond the arbitary map boundary because plant species visited by bumble bees grew on the ground layer under the younger, less dense forest there.

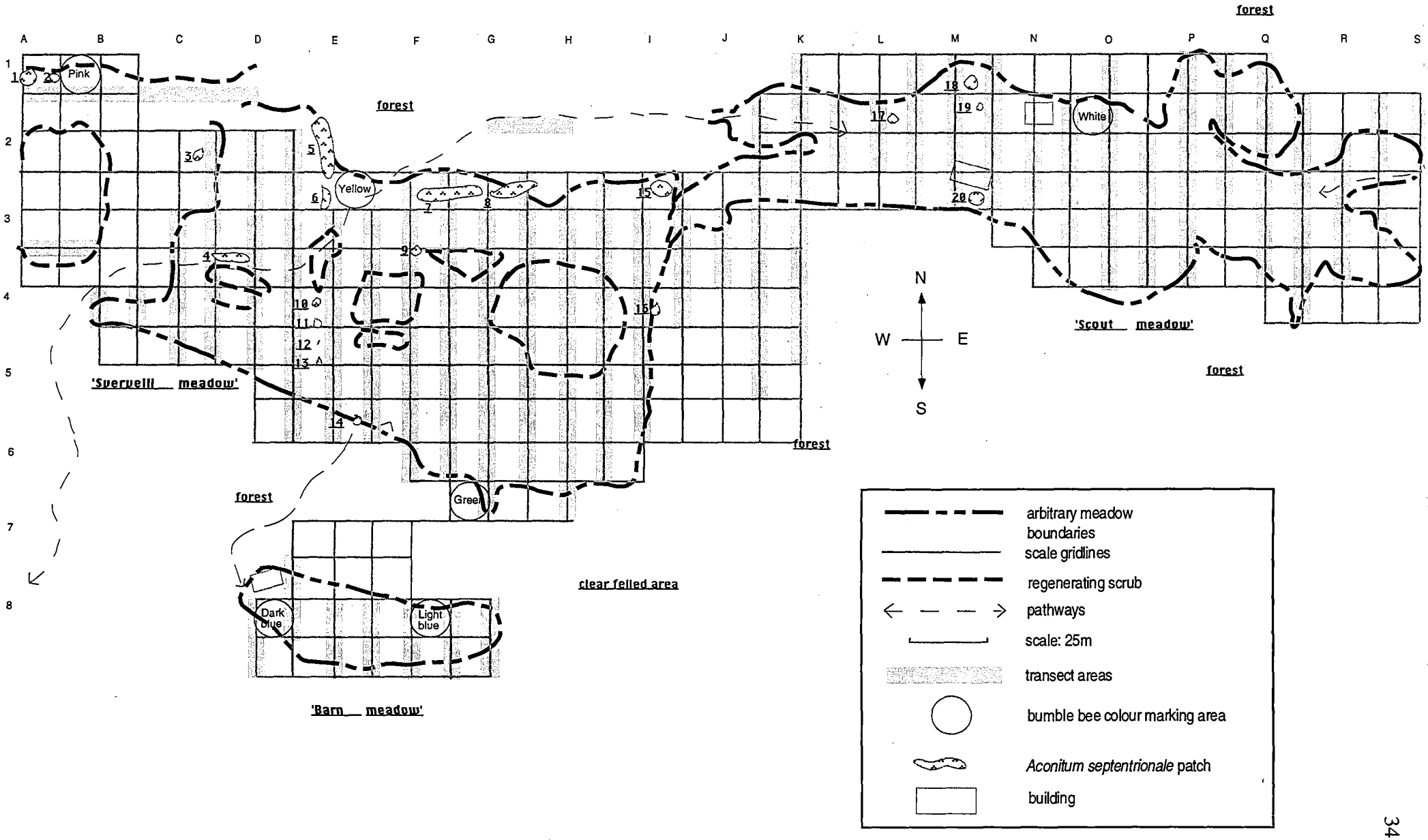


Figure 3.1. The Norwegian meadow study site in Telemark county, south-west Norway. The three meadows and forest between them constituted a total study area of approximately 5 ha.

Plate 3.1. View over Svervelli meadow. Meadows are becoming more separated from each other by encroaching forest boundaries, led by birch invasion. Grass species increasingly dominate in the absence of traditional hay meadow management, under which a wide diversity of flowering herbs flourished.

Plate 3.2. The small barn meadow isolated by thick scrub from Svervelli and Scout meadows. Regenerating birch is visible on the forest edge and in the foreground.

3.3 Movement by individual bumble bees within patches of flowers

3.3.1 Introduction

Pollen flow in flowering plants is a function of pollinator flight distance, pollen deposition schedule and directionality of pollinator flights (Levin *et al.* 1977).

Theoretically, directional movement by pollinators is advantageous for reproductive success of both plant and pollinator populations. It is expected that if bumble bees are foraging optimally (i.e., to maximise energetic gains), they will move directionally to avoid the chances of re-visiting just-emptied flowers (Pyke & Cartar 1992). Directionality also has advantages for the out-crossing of pollen between plants and between plant populations, avoiding inbreeding depression (Levin *et al.* 1971; Handel 1983).

Movement by bumble bees between flowers on protandrous, vertical inflorescences is consistently directional, and is well documented (see Section 2.4.2). However, movement between flowers of "horizontally"-arranged species is not well understood. Waddington & Heinrich (1981) and Ginsberg (1986) concluded that several species of solitary and social bees, including honey bees and bumble bees generally show directionality in their movements in that they tend to fly straight ahead from flower to flower and that moves to the left occur at about the same frequency as moves to the right. However, both random (Zimmerman 1982; Rasmussen & Brodsgaard 1992) and directional patterns of bumble bee movement (Woodell 1978; Levin *et al.* 1971; Pyke & Cartar 1992) have been reported in the literature. Inconsistent results are also reported for bumble bee-movement patterns over whole patches. Random patterns were reported by Rasmussen & Brodsgaard (1992) and directional patterns by Levin *et al.* (1971), Soltz (1986) and Pyke & Cartar (1992).

Several theories have been offered to explain the mechanisms responsible for movement patterns by bumble bees both between flowers and between plants within patches. Differences may be due to the spatio-temporal location of pollen and nectar rewards (e.g., Pyke 1978; Heinrich 1979c; Morse 1980), microclimate effects, particularly wind (Woodell 1978; Pasek

1988), innate behavioural or "external" cues, (for example matching departure directions with arrival ones by memorising arrival directions (e.g., Pyke & Cartar 1992), or departure directions (e.g., Kipp *et al.* 1989), or the ability to recognise and avoid previously visited flowers while habitually moving forward to unvisited nearest neighbour flowers (Zimmerman 1982; Soltz 1986). Ginsberg (1986) suggested that bumble bee directionality is based largely on external cues, and is not merely an artefact of flower distribution. In contrast, others argued that directionality is governed by flower distribution, including plant and flower architecture (see Heinrich 1979e; Zimmerman 1979; Kipp *et al.* 1989; Rasmussen & Brodsgaard 1992). Zimmerman (1982) highlighted potential differences between pollen and nectar-collecting foragers. Also, resolution of scale of measurement and different measurement and analysis methods used may account for some disagreement in results.

Correlating movement patterns with any of the above factors was beyond the scope of this work. Methods of data collection and analysis in the literature vary considerably (Chapter 2.4.2) and are also likely to lead to different results. The most common technique to measure directionality is by series of angular deviations relative to the direction of arrival on a flower or plant by a bee. This method is fraught with computational and analytical difficulties (Kipp 1989), and, from experience in the field, accuracy of recording would be doubtful. Directionality in this work was measured and analysed *per se* using accepted, simple field and analysis techniques (following Woodell 1978, and Zar 1984). Overall directionality was measured because it has most significance for pollen flow. A method of recording directions relative to the recorder, rather than to the bee was used to avoid complications encountered by past authors.

The aim of this section was to test directionality between flowers, and over a patch of flowers on a coarser scale resolution. The hypothesis proposed was that directionality would exist at both scales because theoretically that is the best strategy for plant and bee population fitness.

The need to measure flight distances simultaneously with flight directions

Heinrich (1979c) stated that bumble bees tend to minimise flight distances between flowers because short foraging distances, usually to nearest neighbour flowers or clumps are most efficient in that they increase the time available for collecting more nectar and pollen, hence increasing foraging returns. Rather than simply assuming that all flight distances were equal (as done by Levin *et al.* 1971) flight distances in the following study were measured and compared with nearest neighbour flower distances to test Heinrich's generalisation. Implications for pollen flow based on directional data alone could be misleading if flight distances are highly variable. For example, pollen could travel further after three westward bee moves of 1 m length each, than after 15, eastward moves of 5 cm each. Without the distance information it would be concluded that pollen moved further eastwards which is incorrect, assuming equal pollen deposition and carry-over at each landing.

3.3.2 Methods

Wood cranesbill (*Geranium sylvaticum* L.; Geraniaceae) provides pollen and nectar to a variety of insect visitors including several bumble bee species. Movements by *Bombus lucorum* , a common short-tongued species to visit *G. sylvaticum* were analysed. A large flower patch in the Scout meadow was divided using string, into a grid of 91 one- square-metre cells, and aligned northward (Figure 3.2). A second grid of 2 m x 2 m was positioned within the large grid specifically for between-flower studies.

Overall directionality, defined as the tendency for the majority of flights to be in one direction, was measured by recording a compass direction, relative to the observer, for each move during each individual bee's foraging bout. A "move" was defined in the inter-flower study as the direction taken from one flower to the next, and in the over-patch study as the direction taken from the centre of one square metre grid cell to the centre of the next. Directions were assigned to one of the closest of eight compass points; N, NE, E, SE, S, SW, W or NW. Recording started when the bee entered the grid and finished when it left. Bouts were recorded by dictating onto and audio tape then were later transcribed for analysis. Frequency histograms of flight

distances were constructed for both studies. Distances moved by bumble bees between flowers or grid cells were recorded simultaneously by a second observer to allow uninterrupted observation.

Statistical analysis

Frequency distributions of flight directions recorded were presented in radar plots. Each data set was compared with an expected random distribution using Chi-squared analysis to determine whether movement patterns were significantly random or directional (see Zar 1984). Data was pooled for small sample numbers (< 40 moves/bout) which precluded analysis. Directionality would be represented graphically by a larger peak in one compass direction compared with others on the radar plot, and be supported by a significant Chi-squared test result rejecting the null hypothesis that number of moves in each direction are equal. A random distribution would be reflected by an even spread of compass frequencies in each radar plot direction. Error terms are expressed as mean ± 95 % confidence intervals (95 % C.L.) unless otherwise stated.

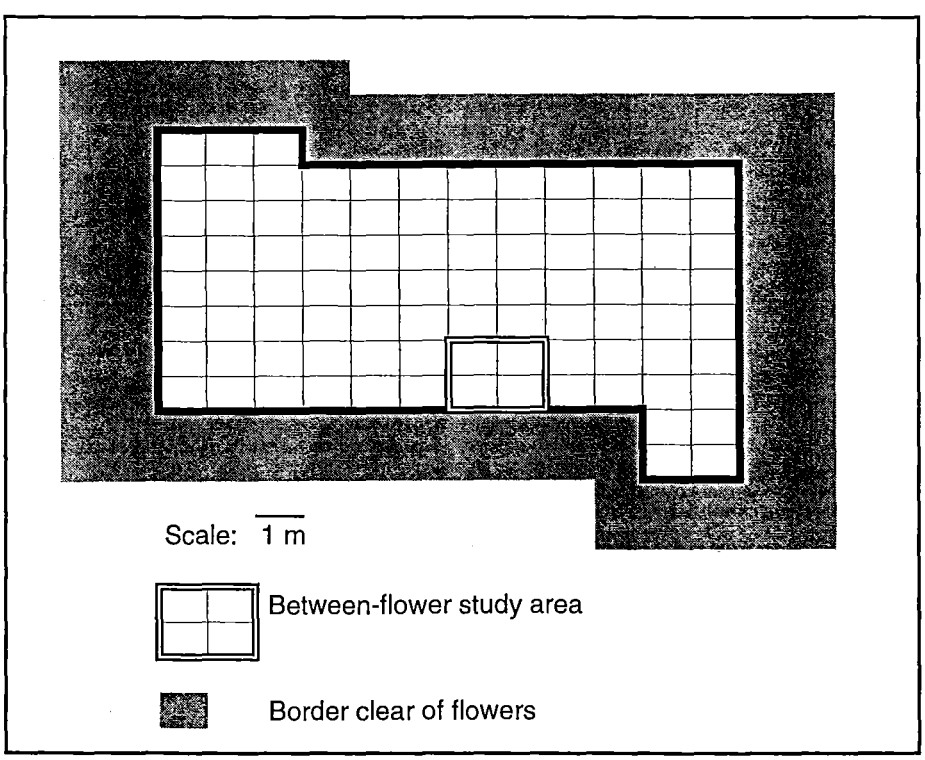


Figure 3.2. Grid array over the *G. sylvaticum* patch in which movement patterns by *B. lucorum* workers were assessed over the patch as a whole, and between flowers.

Movement between flowers

Twenty-six individual foraging bouts by *B. lucorum* workers were recorded between 30 June and 9 July. Each individual was followed through the 2 m x 2 m grid illustrated in Figure 3.2. Nearest-neighbour distances between flowers were measured on 6 July by measuring the distance between each of thirty flowers located at random and their closest flower neigbours in a direct line. Flight distances by bumble bees were estimated to the nearest 5 cm.

Movement over the patch

Twenty-eight foraging bouts by *B. lucorum* workers were recorded between 6 and 14 July through the *G. sylvaticum* patch (Figure 3.2). Foraging paths were recreated from the Dictaphone recordings to assign directions between patch cells. Nearest-neighbour distance between cells was considered to be 1 metre. A frequency histogram of flight distances was produced as in the inter-flower study. Flight distances were categorised to the nearest 1 m.

3.3.3 Results

Overall directionality between flowers

Only five of the 26 individual bouts recorded included enough moves to analyse statistically (Figure 3.3). None of the distributions deviated significantly from a random distribution (χ^2 values for these five bouts were: 14, 7.1, 9.1, 12, 35. $P > 0.05$ in all cases), indicating no directional movement by bumble bees. In addition, all foraging bouts were pooled for analysis (Figure 3.4). There was no significant difference between expected and observed distributions of flight directions ($\chi^2 = 7.5$, $P > 0.05$). Total number of flowers visited per bout in the inter-flower arena ranged from eight to 90. Mean number of flowers visited from all bout data was 27 ± 3.6. The shortest time spent foraging in the arena by any individual was 31 seconds and the longest was 6 minutes 7 seconds.

Overall directionality over the patch

No bouts contained enough moves to analyse as individual bouts. A Chi-squared test on all bouts pooled (Figure 3.4) showed no significant pattern of directionality ($\chi^2 = 14.4$, $P > 0.05$). Total number of moves made to new grid cells per bout ranged from two to 32. Time spent per bout by any bee ranged from 1 minute and 11 seconds to 12 minutes. Total number of flowers visited per bout ranged from 26 to 171. Mean number of flowers visted per bout from all samples was 70.5 ± S.E. 8.60. The actual number of different squares visited ranged from two to 24, indicating several squares were revisited in some bouts.

Flight distances

Mean nearest-neighbour distance between flowers was 5.6 cm ± S.E. 0.81. Figure 3.5 shows the frequency distribution of flight distances between flowers. Of all flight distances recorded, most (62.5%) were less than 10 cm. The single category with the largest number of moves in it was 1-5 cm (containing 32.1% of total moves). Thirty-four per cent of moves were 6-10 cm long. Only 2.6% of moves were 51-100 cm. The longest flight recorded between flowers was ca. 100 cm and the shortest was 1cm.

In the over-patch study, most (88.1% of) of bumble bee moves were to the nearest-neighbour cell 1 m away (Figure 3.5). Moves over 2 m (i.e., over two cells at a time) constituted 7.9% of total moves. The remaining 4% of moves occurred in classes containing longer-distance flights (of 3 m to 11 m). No single longer-distance class accounted for more than 1% of total moves. The longest flight moved in one move between patch cells was 11 m and the shortest 1 m.

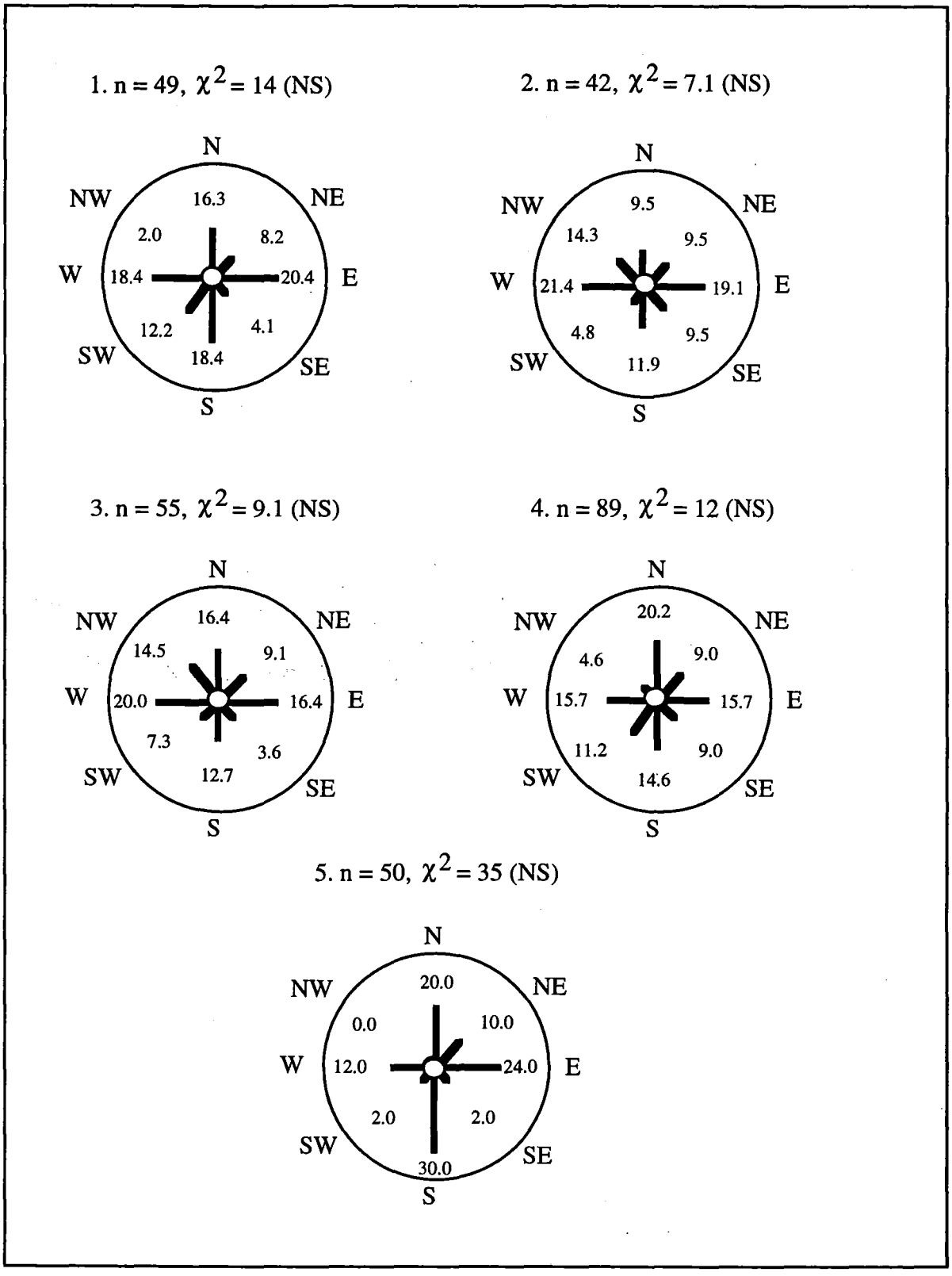


Figure 3.3. Frequency distribution of flight directions for each of five foraging bouts by *B. lucorum* on *G. sylvaticum* flowers. Vector lengths represent percentage of moves in each compass direction. n = total moves per bout. ($P > 0.05$ in all cases).

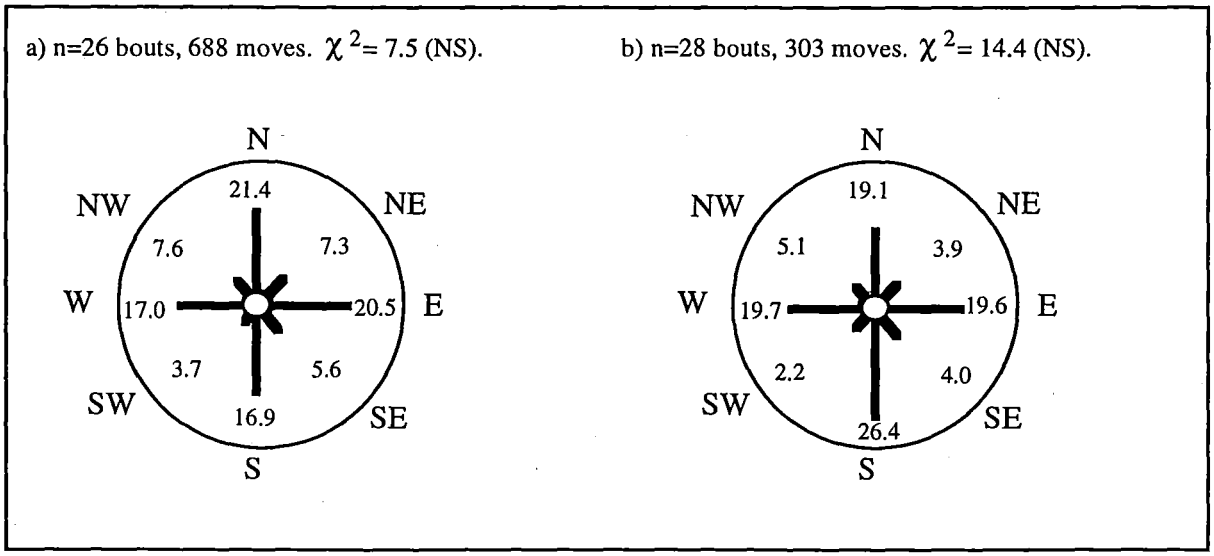


Figure 3.4. Pooled directionality results from several foraging bouts by *B. lucorum* a) between flowers, and b) b) over a patch of *G. sylvaticum.* Radar plots represent frequency distributions of the percentage of moves in each direction. ($P > 0.05$ in both cases).

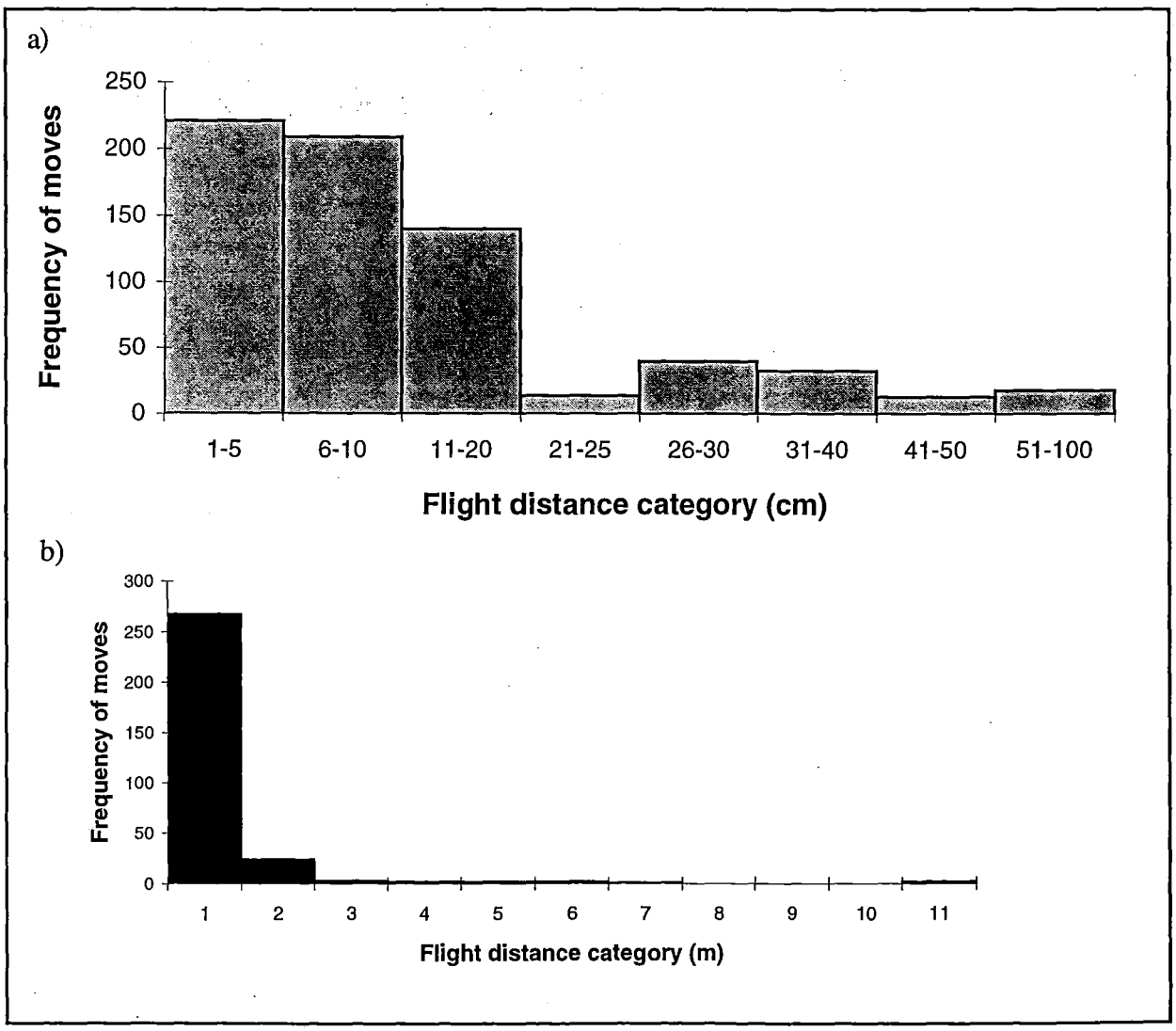


Figure 3.5. Frequency distributions of flight distances for pooled foraging bouts by *B. lucorum* a) between flowers and b) over a patch of *G. sylvaticum.*

3.3.4 Discussion

Random movement by bumble bees between flowers and over the whole patch

All results, whether analysed as individual bouts or from pooled data, indicated consistently random foraging patterns by bumble bees as they moved between flowers, and over the whole patch. This may suggest that resolution of measurement scale is not as critical as expected in determining bumble bee foraging patterns over short distances. It is possible that when data were pooled, directional behaviour by individual bees in the sample may have been masked if they were foraging directionally but each in different directions. For between-flower data, the pooled result is supported by individual bouts also being random. One might expect over-patch bouts by individuals also to be random. However, for over-patch data, no single individual bout had enough moves to analyse statistically. A bigger patch where bees stayed longer and made more moves would be needed to test this using the Chi-squared method.

Bumble bees in this experiment may be exhibiting area-restricted searching behaviour in a patch which is only one part of their foraging route. Once reaching the *G. sylvaticum* patch, in response to encountering a rich resource, bees may have increased their variability of turning angles, which would be reflected as a random distribution. Area restricted behaviour is also characterised by short inter-flower flights with some longer flights, as was seen in this experiment. Pyke (1978) predicted that this behaviour should maximise gains for the forager. Thomson *et al.* (1982) showed that bumble bees in patches of *Aralia hispida* tended to restrict their foraging to limited areas once encountering rich nectar rewards. This behaviour, however, may not be optimal for plant success. To establish whether the *G. sylvaticum* was in fact "rich" would require detailed nectar measurements.

It is not considered that the random pattern of foraging observed was an artefact of the position of flowers or flower clumps. Although *G. sylvaticum* plants formed "clumped" heads, plants were dense resulting in a fairly even horizontal sward. This is reflected in the small error associated with nearest-neighbour flower distances. Heinrich (1979a) suggested that one would generally expect random visitation in clump-arranged species, except when flowers are arranged non-randomly. In an even sward, directionality would be expected. Perhaps the

distribution was not as even as thought at the onset of the experiment. Galen & Plowright (1985) attributed bumble bees' consistent directionality on spike flowers as an evolved behaviour in response to the highly predictable reward sequence and architecture. *G. sylvaticum* as a horizontally-arranged species is not as ordered architecturally, and therefore rewards are probably not as predictable to bumble bees.

Potential implications for plant reproductive success of random foraging

At the small scale between flowers and within a patch, directionality is important for increasing the probability of cross-pollination between self-incompatible plants. Although random foraging will result in some crossing, directional behaviour is probably more reliable. Individual behaviour may not be so critical if several pollinators are visiting the patch, and between them, sufficient pollen cross-over may be secured. Detailed analysis of pollen carry-over (Levin *et al.* 1971) and a reproductive-success measure such as seed production in relation to bumble bee visitation patterns is necessary to determine whether pollination is actually limited by pollination shortfalls, or whether other mechanisms are responsible for plant declines through inbreeding depression. Other mechanisms of a plant's breeding system should be considered in evaluating its likely success; whether it has other biotic (insect or animal) pollinators or seed dispersers or can rely on abiotic pollination methods (wind, water). Some plants may also have a vegetative reproduction strategy (e.g., clover stolons). Perhaps it is an adaptive strategy of *G. sylvaticum* to offer sparse rewards from its shallow flowers. This may encourage more longer-distance flights between patches by bumble bees when they leave because of low rewards. With many visitors and being the largest patch of flowers in the area, pollen and nectar rewards are likely to be low at times of the day with high insect visitation.

Significance of directionality at small and large scales

Directionality on a larger scale between patches may have more significance for the success of plant gene out-crossing to different plant populations. On a large scale, plant patches tend to be very patchy and non-evenly distributed. If pollinators forage directionally, they may "miss" these patches. Pyke & Cartar (1992) described advantages and disadvantanges of directionality at different scales. Although random foraging within a patch can increase the risk of revisiting empty flowers, on a large scale in a patchy environment directionality can increase the risk of

missing patches of high quality because the patches are almost always unevenly distributed. Random patterns of movement may therefore be a greater advantage for gene cross-over between patches. It may be possible that bumble bees move directionally until encountering a rich area, then intensify their search effort, increasing turning angles, which in turn would result in randomness in area-searching-type behaviour as is found in predator-prey search strategies. Soltz (1986) stated: "After visiting a poor flower or sparse patch, bees should exhibit basically straight paths with little angular deviation and move greater distances between inflorescences to increase their chance of reaching a richer patch or part of a patch where increased turning angles can be expected as the bumble bee restricts it searching area". Soltz tested this theory which was proposed by Pyke (1978) based on his model, suggesting that the richness of previously visited inflorescences causes area-restricted foraging behaviour in bumble bees. Soltz (1986) found no evidence for it, or no correlation with plant richness or density. She concluded that directionality by bumble bees is largely a result of their habit of visiting nearest-neighbour flowers, and having an ability to recognise and avoid just-visited flowers.

Explanations for random foraging strategies ; theoretical implications for bumble bee success

Zimmerman (1979) recorded random foraging for *B. bifarus* Cresson and *B. flavifrons* Cresson on *Potentilla gracilis* (L.; Rosaceae), a horizontally arranged plant architecture comparable to *Geranium* spp. rather than a spike architecture on which much of the directional literature is based. He concluded, as predicted by an optimal foraging model, that random movement by pollen-collecting bumble bees is the optimal strategy in terms of energy gains when "the probability of revisitation of flowers in a patch is low", and the penalty from revisiting a flower is low. In other words, when there is not much to gain from flowers, bees can forage randomly without wasting too much energy. The risk of encountering successive low-rewarding flowers is minimised. Conversely when there are flowers rich in reward, the probability of bees' visiting them is high. The optimal strategy should be an organised directionality for the bumble bee to maximise profits. Similar strategies in foraging patterns have been reported for bumble bees, leaf cutting bees, honey bees and carpenter bees (Levin *et al.* 1971), butterflies (Levin *et al.* 1971), and nectar-feeding birds (Cody 1971). Zimmerman (1982) concluded that bumble bee foraging behaviour is highly flexible depending on the spatial

arrangement of nectar and pollen rewards. In addition to flexibility, bumble bees are flexible in relation to reward level. On previously-screened plots of *Vicia cracca* L. and *Trifolium pratense*, Morse (1980) found that bumble bees concentrated foraging effort (spent longer in patches and visited more flowers) in areas with high nectar. Zimmerman (1982) did not record area-restricted searching behaviour and suggested that bumble bees can assess rewards while flying. This would save them wasting time and energy by landing and probing flowers which contain little or no pollen or nectar.

Minimisation of flight distances

The generalisation by Heinrich (1979a) that bumble bees visit nearest-neighbour flowers is supported in this work at both small scales. In this thesis study, the majority of flights in both cases were in the nearest-neighbour range. Nearest-neighbour distance between flowers was 5.6 ± 0.80 cm, and the majority of flights between flowers were less than 10 cm long (62.5%). The single largest category of flights (32.1% of total moves) were 1- 5cm long. That most (88.1%) of flights went to the nearest 1 m size cell in the coarser scale work in this study could partly indicate an artefact of method. Very few large moves were made (< 4% in 3 m to 11 m range). Short flight distances are characteristic of area-restricted searching behaviour to maximise profit when searching in rich-reward patches (Pyke 1978). Heinrich's (1979c) work on white clover showed that sharp angular turns by foragers reflected rich nectar rewards. Perhaps this is the mechanism operating in the *G. sylvaticum* patch.

Limitations of this work and future aspects for study

In this work, directionality was not correlated with any biotic or abiotic factors and discussions have been largely theoretical. In order to isolate different factors which may control foraging patterns observed, artificial arrays in which variables can be controlled would be useful. Most arguments have been in terms of the spatio-temporal rewards. Flowers should be screened to exclude other insect visitation to make rewards even in space and time (as done by Morse 1980). Insects should ideally start at the same point and go through the same array. Nectar and pollen rewards should be accurately measured and correlated with observed flight patterns. "First-approached" and "last-faced" directions could be recorded for all foragers to test innate-behavioural habit-based theories. It is possible that patch shape influenced flight patterns if bees

concentrated foraging along edges. Closer examination of the reconstructed flight paths over the patch would be needed to assess possible edge-effects, which could result from easier physical access by the insect, and better pollen and nectar rewards in the absence of competition for light and moisture on all sides from other plants.

Some very complicated models exist in the literature which are based on benefits for the bee through optimal foraging theory. In some cases, directionality by bumble bees has been recorded which could not be explained in terms of such energy profit models (e.g., Hodges & Miller 1981; Soltz 1986). The role of other mechanisms in determining bumble bee foraging patterns should be examined in more detail, particularly external factors, both innate and abiotic. Wind and sun angle, which are known to guide honey bee orientation, should be profitable areas for future research. Pasek (1988) reported that wind and wind breaks in landscapes have a major influence on flying insect patterns. Woodell (1978) demonstrated strong correlations between flight directionality and wind direction on patches of Sea-lavender *Limonium* sp. and Thrift *Armeria maritima* (Miller) (Plumbaginaceae). Work in this chapter was carried out on calm days only, so this aspect could not be compared with Woodell's work. Woodell concluded that bees track up an odour plume to forage, and can land more easily going into the wind. It was demonstrated that flight direction reversed with wind direction. If vegetation boundaries of regenerating forest in the meadows in this Norwegian study are restricting windflow, there may not be strong odour plumes to guide bees. Wind probably is a major factor affecting directionality, but because other authors have found directional foraging in the absence of wind, it is probably not the only factor affecting it. It would be interesting to repeat this experiment in windy and calm conditions. Detailed microclimate records of windspeed and direction would be needed. Patterns of foragers could also be influenced by the presence of other individuals through direct and indirect competition effects. Holmes (1961) reported that the presence of honey bees "dissuaded" bumble bee foragers. Competition effects are another area warranting further research.

In summary, it is not clear what mechanisms control movement patterns in bumble bees, and without further research what the significance of random movement to bee and plant populations is on a small scale. No single aspect has been clearly shown consistently to control

directionality in bumble bees foraging within patches in the literature. The work done in this chapter highlights the multivariate factors which influence foraging behaviour. Cues could be plant-, bee- or environmentally-mediated or a combination. More research is needed to identify the relative importance of each aspect. Artificial arrays where each aspect can be controlled will be valuable for this.

In conclusion the random foraging observed in patches of *G. sylvaticum* may be explained by one of two theories); 1. that bumble bees are exhibiting area-restricted searching behaviour. The *Geranium* flower patch may have been a relatively rich section along their foraging route. In the meadow it was a very dense single stand of resource . Once encountering the patch, bees increased turning angles and variability of turning (reflected in a random distribution of foraging directions) and minimised flight distances; or 2. the resource did yield sparse rewards (which is possible as there were many other visitors to the patch), and according to the optimal foraging model proposed by Pyke (1978), being random in sparsely rewarding patches can be the optimal strategy for energy profit by bumble bees.

3.4 Movement by bumble bees between patches and between meadows

3.4.1 Introduction

Large scale studies of bumble bee movement in fragmented semi-natural habitats

There is a shortage of large scale studies of bumble bee movement. Most records are anecdotal and part of projects with a separate focus, being chance reobservations with sampling not organised in a replicated way. This shortage is probably because of the difficulties involved in organising and carrying out work in the field at a large scale. Recently more authors have addressed large scale bumble bee movement in modern fragmented agricultural habitats (Banaszak, 1983; Dramstad 1996a, Saville 1996). Excluding Bowers (1985) in the US and Kwak *et al.* (1989) in The Netherlands, there have been no concentrated efforts in semi-natural or natural habitats on a large scale to measure bumble bee movement. Such studies are vital in understanding pollinator efficacy in areas of conservation interest threatened by plant and bee

species decline through effects of habitat fragmentation through land-use intensification or abandonment. Interest in the value of the type of research is increasing the UK, US, and other parts of Scandinavia.

This study provides basic data on bumble bee mobility within a typical, abandoned Norwegian herb-rich meadow system of conservation value (see Section 2.3.1; 3.1 and 3.2 for site description). In the future it is hoped this data will eventually be of value in the development of managment strategies to preserve the unique floral diversity, and of the many species of bumble bee pollinators which depend on the plants for their own survival.

Patterns of pollinator movement within the meadow system will have implications for pollen flow between different plant populations. If bumble bees are highly mobile between patches and meadows then theoretically gene crossover through pollen transport between different plant populations will be high, maintaining a high level of genetic stability. This work considers and discusses the potential roles of physical landscape features, bumble bee behaviour, and resource distribution in relation to patterns of movement detected. Experimental testing in relation to these factors however, was beyond the scope of this work.

There is evidence that physical landscape features, such as hedges and forest edges affect movement of ground-dwelling predatory insects (Mauremootoo *et al.* 1995) and of flying insects (Bowden & Dean 1977; Fry 1993a). Plowright & Galen (1985) found that bumble bees are more likely to move rapidly through uniform areas than those with boundary or landmark features, although the mechanisms could not be explained. Fry & Robson (1994) experimentally demonstrated impeded butterfly movement across artifical barriers as low as 1 m tall. Hover fly movement was also shown to be restricted by hedges (Evans 1996). It is possible that regenerating forest boundaries dividing the Norwegian meadows act as a barrier to pollinator movement.

Specific aims of this section were;

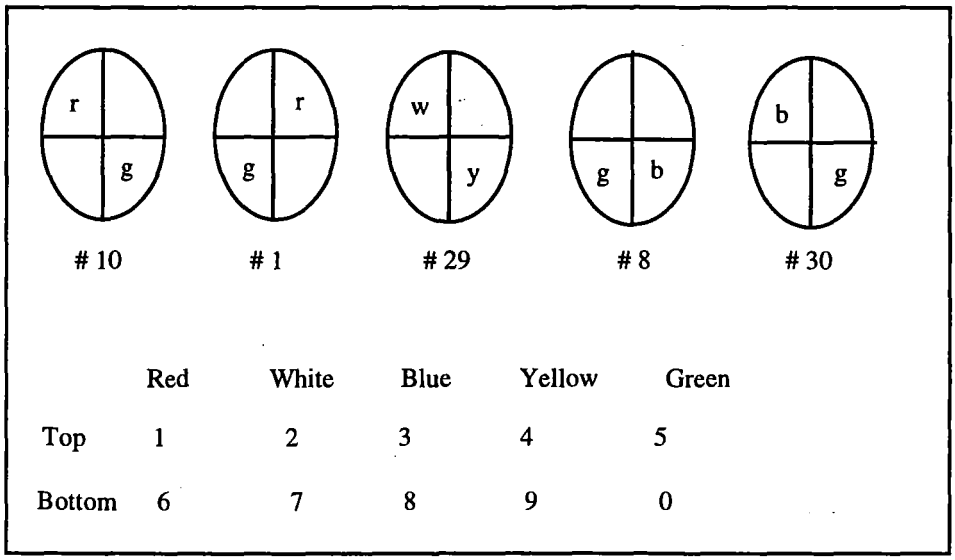
- to test mobility of bumble bees between distinct patches of one plant species
- assess the loyalty of bumble bees to forage patches in which they were marked
- test whether bumble bees crossed between meadows, and if so the extent of it.

3.4.2 Methods

Marking and reobservation of bumble bees between patches of one plant species

Twenty distinct patches of Northern wolfsbane *Aconitum septentrionale* , distributed throughout the meadow system were mapped to assess bumble bee movement between patches of the same plant species on a large scale (Figure 3.1). *A. septenrionale* is a typical "bumble bee-plant" occurring in woodland and alpine areas (Løken 1949). It is visited by one highly specialised bumble bee species (*B. consobrinus* Dahlbom) as well as by some generalists (Mjelde 1983; Thostesen 1993). It has many large complex shaped, blue flowers arranged in spikes and offers good pollen and nectar supplies which require a bumble bee's strength to access them legitimately.

Between 16 and 18 July, 30 bumble bees were code-marked individually. Each individual received a unique series' of coloured spots in specific thorax positions encoding a specific number (Figure 3.6). This method was demonstrated by Dr. M. Kwak (pers. comm.). Bees were caught using sweep nets and transferred to plastic and gauze marking tubes. Bees were held in the tube with a sponge holder against the gauze and dotted from the outside with the appropriate spots of paint (Tippex® or acrylic paint) using a fine brush or skewer stick. After briefly waiting for the paint to dry bees were released. Twelve of the 30 individuals marked were *B. pratorum*, 9 *B. lapidarius* (L.), 6 *B. hortorum*, 2 *B. lucorum* and 1 *Psithyrus* species. Twenty out of thirty marked were worker caste. All bees were marked in patches in Svervelli meadow except for the first 3 individuals which were marked in the Scout meadow. Most bees were marked on patches 4 (14 marked), 7 (7 marked) and patch 8 (5 marked) in the centre and top of Svervelli meadow (Figure 3.1).



	Red	White	Blue	Yellow	Green
Top	1	2	3	4	5
Bottom	6	7	8	9	0

Figure 3.6. Examples of marking codes used on individual bumble bees foraging on patches of *A. septentrionale*. Each oval represents a dorsal view of a bumble bee thorax divided into quarters. Colour spots are read left to right and assigned table values according to their upper or lower position.

Five reobservation sessions for individually marked bumble bees were carried out on 25 July, 27 July, 28 July, 1 August and 2 August 1993 respectively. Each session involved a 20-minute circuit walk around the patches in the same order each date, starting at the patches in the left top corner (facing north) of Svervelli meadow and finishing in the top right end of the Scout meadow.

The number of flowering spikes per patch was estimated to the nearest five on 25 April (except in patches with fewer than five flowering spikes in total). Totals ranged from single spikes in patches 12 and 17, to approximately 130 spikes in patch 8. Each spike contained approximately 7 flowers on average over the week-long study period. Patch 8 was estimated to contain a mean of over 900 flowers. Three patches contained between 100 and 130 flowering spikes (numbers 4, 5, and 8). Three contained between 40 and 100 flowering spikes (numbers 1, 6, and 7). Five had between 10 and 20 flowering spikes (numbers 2, 9, 14, 15, and 20). Nine patches contained between one and five flowering spikes (numbers 3, 10, 11, 12, 13, 16, 17, 18, 19). The relative areas covered by patches and all patch positions in the meadow system are mapped in Figure 3.1.

Marking bumble bees within selected areas to assess between meadow movement

Several species of bumble bees were marked on four days between 16 and 19 July within each of the six assigned marking areas (Figure 3.1). Areas rich in floral supply were selected where highest numbers of bees could be marked. A total of 260 bumble bees were marked in several sessions, with effort divided equally for each area on each day. Bumble bees were caught within an assigned 12.5 m diameter circle and marked using the same netting and tube method as in the individual bumble bee study between patches. Colours assigned to areas were as follows; dark blue, green, light blue, pink, white or yellow (Figure 3.1). Figure 3.7 and Table 3.1 summarise bumble bee marking information including species, caste, and plant species on which bumble bees were foraging when caught to mark.

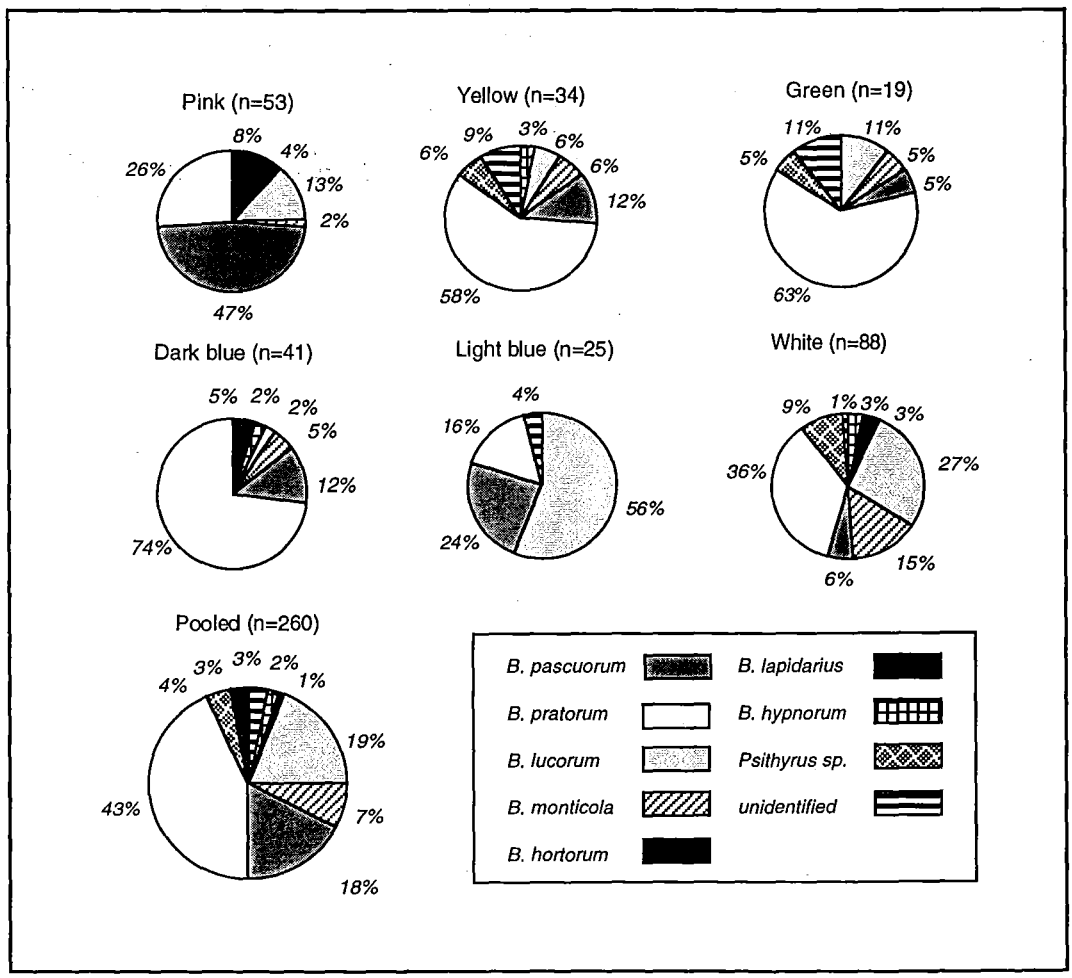


Figure 3.7. Breakdown of numbers and species of bumble bees marked in each of six differently colour-coded marking areas within the meadow system, and pooled data for all areas combined.

Table 3.1. Proportions of bumble bees marked from differently colour-coded meadow areas, and the range of plant species on which they were caught.

Percentage of bumble bees marked from each plant species:

Plant species	Pink (n=53)	Yellow (n=34)	Green (n= 19)	Dark blue (n=41)	Light blue (n=25)	White (n=88)	% of total marked (n=260)
Aconitum septentrionale Koelle		2.9					0.4
Campanula rotundifolia L.				2.4			0.4
Campanula sp.						1.1	0.4
Geranium sylvaticum L.	9.4	67.6	78.9	75.6	16.0	51.1	47.3
Geum rivale L.		2.9	10.5				1.2
Hieracium sp.					4.0		0.4
Hypochoeris maculata L.	1.9					1.1	0.8
Lathyrus pratensis L.	9.4						1.9
Lotus corniculatus L.				2.4			0.4
Lychnis viscaria Bernh.			5.3				0.4
Melampyrum pratense L.	1.9	23.5			80.0	1.1	11.5
Rubus idaeus L.				14.6			2.3
Silene sp.				2.4			0.4
Solidago virgaurea L.	1.9						0.4
Trifolium spp.						39.8	13.5
Vicia sp.	73.6						15.0
Reobserved flying	1.9	2.9	5.3	2.4		1.1	1.9
Unidentified						4.5	1.5

Percentage of bumble bees marked in each caste:

Caste	Pink	Yellow	Green	Dark blue	Light blue	White	% of total marked
Worker	85	68	58	71	92	47	66
Male	8	26	32	29	8	52	30
Queen	8	3					2
Unidentified		3	11			1	1

Reobservation of bumble bees marked in the six areas in the meadow system

Transects were divided into 204, 12.5 m sections marked by labelled stakes in the field. Transect sections were divided onto six small scale-maps of the area. Each observer was assigned a different section. Observers walked at an even as pace as possible (approximately 2.5 minutes per 12.5 m section) while observing and marking bumble bee details within 2.5 m of either side of the transect line. Each reobservation period lasted ca. 1 h 30 min with some observers finishing earlier because they had a smaller area to cover. At least four observers carried out each reobservation. Seven observation walks were carried out: 19 July (2 sessions), 20 July (2 sessions), 27 July, 28 July and 8 August. Details recorded were bumble bee species, caste, map location, and plant species they were foraging on. The distance bumble bees were reobserved from their marking points was measured manually later from maps produced to scale in the field. Accuracy of recording is estimated at approximately ± 3 m for each point marked on the scale map.

3.4.3 Results

Bumble bees marked on patches of *A. septentrionale*

Thirty individuals were originally marked. In total, 25 reobservations of these were made. Only 18 of those reobservations were of recognisable codes. The remaining seven were unreadable. Either bees moved too fast to read, or part of the spot combination had worn or been groomed off. Five individually marked bees which were observed during meadow transects walks which intersected *A. septentrionale* patches were added to the 13 individually marked bumble bees reobserved on the wolfsbane circuit walks. Some multiple observations occurred. Eleven different individuals were reobserved. Five individuals were seen twice and one individual observed three times. Three of the individuals seen twice were on different patches to those on which they were marked. Eight were reobserved on the same patch on which they were marked. Three were found on nearest neighbour patches. No individually marked bees were observed beyond Svervelli meadow. Figure 3.8 shows the flight distances recorded for reobserved bumble bees.

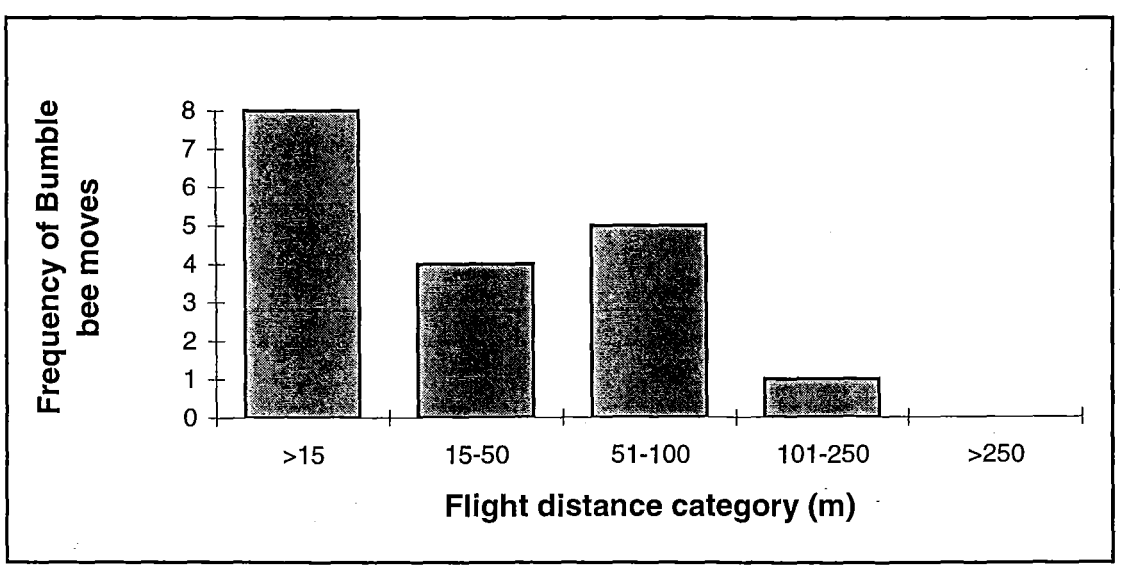


Figure 3.8. Frequency distribution of flight distances from 18 reobservations of individually marked bumble bees between patches of *A. septentrionale.*

Between-meadow reobservations

Figure 3.9 and Table 3.2 summarise reobservation information; bumble bee colour, caste and species, and plant species on which reobservations were made. At the first reobservation on 19 July, nearly 200 bees had been marked. Numbers marked overall in areas ranged from 10 in the "light blue" Barn meadow area to 85 in the "white" Scout meadow area.

Figure 3.10 summarises the frequency distribution of flight distances for all bumble bees reobserved (n=109). Most bees (87 %) were reobserved 50 m or less from their marking point. 42% were reobserved 15 m or less from their marking point, i.e., almost within the 12.5 m diameter circle in which they were marked. Eight per cent were found 51-100 m away, 3% at 101-250 m away, and only 2% at 250 m or greater away. There were only five records of bees moving 100 m or greater away and crossing between meadows (Table 3.3). Most bumble bees left the marking circle in which they were marked (85% on average), but 84% on average remained in the meadow where they were marked. The maximum flight distance recorded was ca. 320 m.

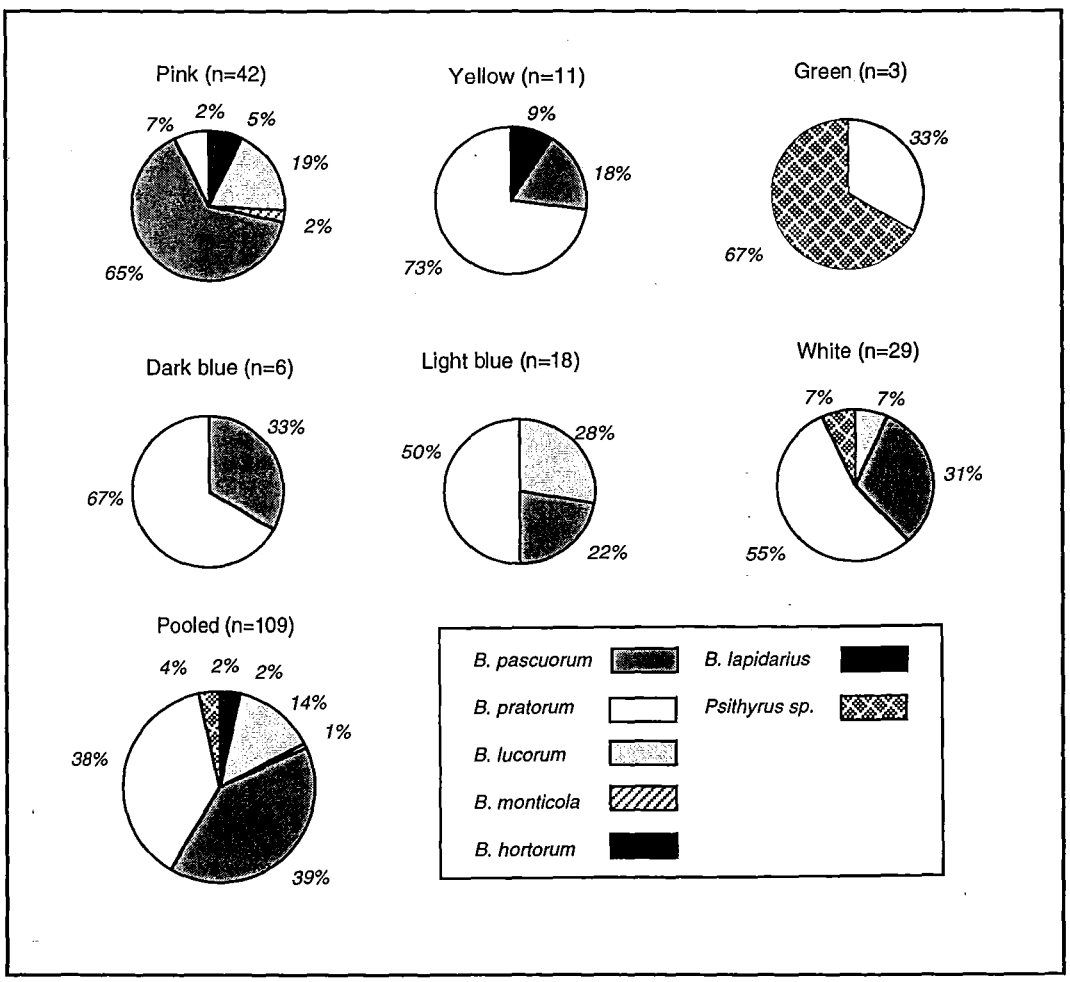


Figure 3.9. Breakdown of bumble bee numbers and species reobserved following marking

Table 3.2. Breakdown of reobservation data for marked bumble bees including species, caste, colour, and flower species on which bumble bees were observed.

Percentage of bumble bees reobserved on each plant species:

Plant species	Pink (n=42)	Yellow (n=11)	Green (n=3)	Dark blue (n=6)	Light blue (n=18)	White (n=29)	% of total reobservations (n=109)
Centaurea jacea L.			33.3				0.9
Cirsium sp.						3.4	0.9
Epilobium angustifolium L.		9.1					0.9
Galeopsis sp.						3.4	0.9
Geranium sylvaticum L.		63.6	33.3		11.1	55.3	23.9
Hieracium sp.						6.9	1.8
Hypericum sp.	2.4						0.9
Knautia arvensis (L.) Coulter			33.3				0.9
Melampyrum pratense L.	17.1			50.0	72.1	6.9	22.9
Rhinathus minor L.					5.6		0.9
Rubus idaeus L.					5.6		0.9
Solidago virgaurea L.				33.3	5.6		2.7
Trifolium spp.	2.5	9.1				20.7	7.4
Vicia sp.	78.0	9.1					31.2
Reobserved flying		9.1		16.7		3.4	2.8

Percentage of bumble bees reobserved in each caste:

Caste	Pink	Yellow	Green	Dark blue	Light blue	White	% of total reobserved
Worker	86	64	33	83	78	18	74
Male	2	36	67.0	17	22	6	17
Queen	5						2
Unidentified	7					5	7

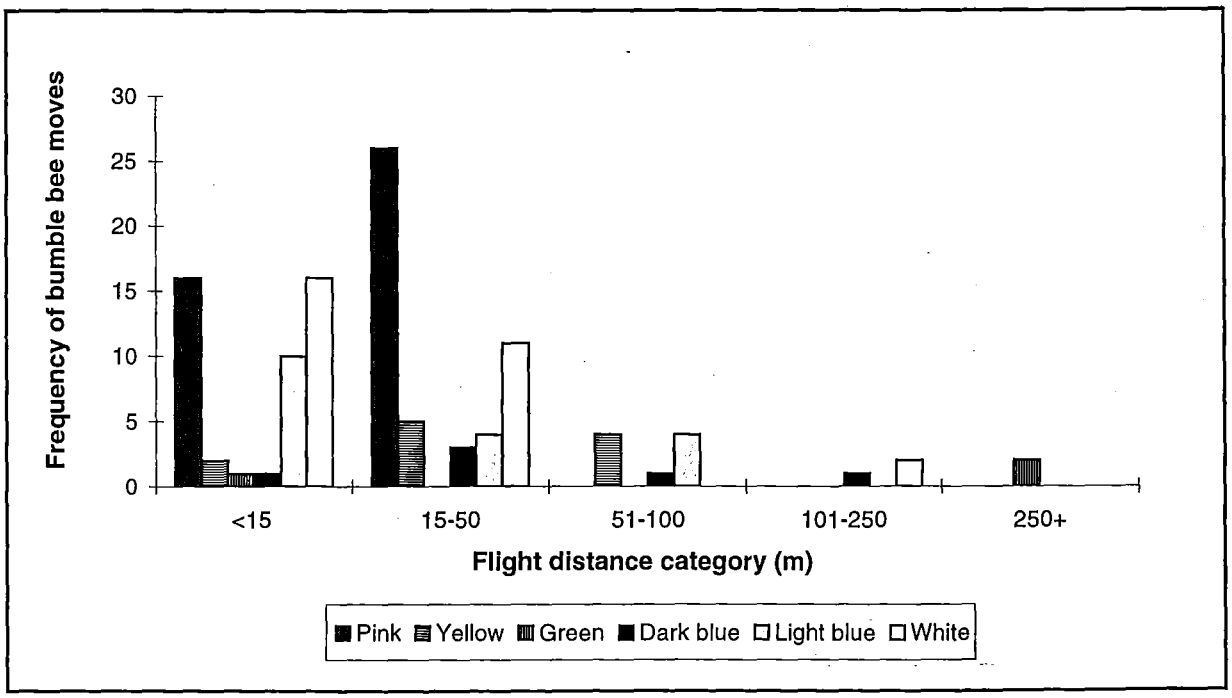


Figure 3.10. Frequency distribution of flight distances for all bumble bees reobserved in six meadow areas in transect walks after marking.

Table 3.3. Bumble bees which crossed forest boundaries to different meadows during a large-scale mark-reobservation project.

Species	Caste	Colour mark	Distance travelled (m)	Meadows travelled between
Bombus lucorum	Male	White	129	Scout-Svervelli
Bombus pratorum	Worker	Dark blue	128	Barn-Svervelli
Bombus pratorum	Worker	White	290	Scout-Svervelli
Psithyrus sp.	Male	Green	278	Svervelli-Scout
Psithyrus sp.	Male	Green	321	Svervelli-Scout

Loyalty of bumble bees to areas in which they were marked

On average, for all colour groups, 85% of bumble bees left the circle in which they were marked. However, 84 % of marked bumble bees remained in the same meadow in which they were marked. Overall, regardless of colour group, 104 of the 109 reobserved marked bumble bees did not cross between meadows (95.4%). Figure 3.10 highlights how most bees were reobserved 50 m or less from their marking point. It is not possible to say how representative the reobserved bees' behaviour was. Multiple reobservations were likely to have occurred.

3.4.4 Discussion

Non-specificity of bumble bees to *Aconitum* patches

The individual marking method for *Aconitum* patch-marked bees had limited success. Missing colour codes which bees may have groomed off, and certain individuals moving too quickly resulted in reduced reobservation number because of unrecognisable codes. The most common visitors were short-tongued generalist species which also foraged on other meadow flora including *B. pratorum, B. lapidarius* and *B. lucorum.* Visitation by the specialist bumble bee *B. consobrinus* described by Thostesen (1993) in a nearby western Norwegian site was not confirmed in these meadows. Short-tongued species foraged mostly for pollen, but robbed nectar through corolla bases, as found by Thostesen (1993). The long-tongued *B. hortorum* reached the nectaries hidden in the elongated hood legitimately. The specialist *B. consobrinus* may depend on *A. septentrionale* as claimed, but it appears from this work that the plant does not depend on any one bumble bee species for pollen transfer. Because few individuals foraged

solely on *Aconitum* (some were seen on other plant species), it might suggest that *Aconitum* is a minor plant in "traplining" sequences (see Section 2.2.2) and certain patches will be revisited predictably. This is supported from multiple observations of exactly the same individuals on the same plants on consecutive days. This behaviour, and the fact that most bees stayed on or went to the nearest neighbour patches, may limit pollen flow between patches, so although beneficial for the bees (as it saves time searching afresh each bout) may not be optimal for plant success. However, the critical information to gather is the threshold level of pollen transfer that is required to maintain plant population genetic stability. Rasmussen & Brodsgaard (1992) showed on patches of *Lotus* sp. that only 2.6% of pollinator flights between patches resulted in a considerable level of out-crossing. The work of Thostesen (1993) which measured pollen removal and deposition rates between flowers of *Aconitum* by *B. consobrinus* and tested corresponding measures of male fitness would be useful extended to a larger scale between patches. Future work could test whether *B. consobrinus* visits solely*Aconitum* plant patches. This could be approached by assessing pollen loads in addition to field observations. Fluorescent powders dusted on flowers and picked up by foraging bees is a useful tool to trace movement patterns (Thomson *et al.* 1986). This could be a useful technique to use in future studies between patches in the Norwegian meadows. Patches could be searched for powder using hand-held ultraviolet lights at night.

Loyalty of bumble bees to areas in which they were marked.

Species composition and number of reobservations made were higher than expected by the transect method over such a large area. A total of 260 bees was marked in the six colour-coded areas and 109 reobservations were made. Because individuals were not identified it is probable that multiple reobservations occurred. The most common species marked and reobserved were *B. pratorum*, *B. lucorum* and *B. pascuorum*. Results are partly biaised because more bees were marked in some colour areas than others, and the plants in them influenced the bee species composition. In particular, the pink area consisted of mostly *Vicia* spp. and *Trifolium* spp. (the most concentrated patch of long-corolla tubed flowers in the study site) on which the long-tongued *B. pascuroum* and *B. hortorum* foraged. More bees were marked in the pink area than in other areas. A large proportion of markings and reobservations were also carried out in the white marking area in the Scout meadow. The predominant visitors were short-tongued *B.*

lucorum and *B. pratorum*. Several reobservations were made on *Melampyrum pratense* (L; Schrophulariaceae) and species different from those on which they were marked, indicating mixed-species foraging patterns.

The restricted nature of bee movement observed may reflect area-restricted searching strategies by bumble bees. The tendency of bumble bees to "major and minor" i.e., concentrating foraging efforts on one major species, while constantly sampling a range or flower species, could also explain the patterns of movement observed. Theoretically, once bumble bees discover a rich resource they should continue to exploit it. The marking areas were very concentrated areas of resource with many bumble bee visitors.

This work supports evidence that bumble bees can be very loyal to certain forage patches. "Forage-patch fidelity" has been recorded by several authors in various habitats and is thought to reduce uncertainty in food intake caused by spatial heterogeneity (Heinrich 1975). Saville *et al.* (1996) also found in a patchy agricultural landscape in southern Norway that bumble bees marked on patches were very loyal. Reobservation rates were highest within 50 m of marking points, with several within 25 m. A maximum flight distance of ca. 300 m was recorded in their work, similar to that recorded in this experiment (which was ca. 320 m for a *Psithyrus* sp. and ca. 290 m for a *B. pratorum* worker). Figure 3.10 showed that most bumble bees (87%) travelled less than 50 m. In this work, 42% of bumble bees reobserved were recorded less than 15 m from their 12 m diameter marking areas. Bumble bees can predictably revisit the same patches during "traplining" behaviour (see Section 2.2.2). These predictable sequences may be advantageous to the bees who save time and energy searching each time they leave the nest by utilising this behaviour, but benefits for plants may be limited in terms of potential pollen cross-over between different plant populations. In studies of bumble bee visitation to meadows containing the rare dark purple rampion *Phyteuma nigrum* (Schmidt.; Campanulaceae), Kwak *et al.* (1989) recorded bumble bees moving a maximum distance of ca. 250 m between meadows in the Netherlands. However, these results are highly dependent on reobservation effort and probably the spatial distribution of resources, and are therefore impossible to compare between sites. One should interpret results from transect-based reobservation work with caution because of the decreasing probability of reobservation with increasing distance

from marking point (see Section 2.4.2). No mathematical correction was made for this aspect in this work.

There is no doubt that bumble bees are capable of flying several hundred metres. They have large honeystomach capacity and are very robust. What is important is how far they do actually fly rather than what they would under exceptional circumstances, and how this relates to the success of plant species.

Relationship between resource and bumble bee distribution

Without mapping all the plant resources it is impossible to judge whether the bumble bee distribution actually matched plant distribution. Certain bumble bee species were concentrated consistently in some areas. The long-tongued specialist *B. pascuorum* was concentrated in the pink area where the highest concentration of long corolla-tubed*Vicia* spp. and *Trifolium* spp. existed in the study area. It is the specialist pollinators that are especially vulnerable to habitat fragmentation effects (Rathcke & Jules 1993). Because they are not generalists they can not switch to other plants, whereas short- and medium-tongued species are more flexible and opportunistic in their foraging habits. Artficially manipulated patch experiments such as the "farmland ecology patch experiment" at Rothamsted Experimental Station in the UK (Palmer & Munden 1993), and a similar patch dynamic experiment at the University of Kansas (Holt *et al.* 1993) will help to elucidate effects of spatial dynamics on insect movement on plant populations more accurately.

Minimal crossings by bumble bees between meadows

That only five bumble bees out of 260 marked in total crossed between meadows may be a result of restricted movement governed by innately-controlled foraging strategies like patch fidelity or loyalty, or because of physical landscape features obstructing their movement.

It is possible that forest edges act directly on bumble bee movement as physical barriers. It is also possible that they act indirectly on bee movement by changing the meadow microclimate . Tall forests influence microclimate. They shade, change wind and temperature altering conditions for plant resource availability. Also wind directly affects many flying insect species (Pasek 1988). Bumble bees are strong fliers, so they may fly over the forest. The sloping

nature of the meadows would mean reduced flying distances compared with direct flight through the forest. However odour and visual cues from isolated clearings could be missed, if blocked by dense forest. In areas of the meadow system where edges were not dense, bumble bee-visited plants grew on the ground layer (e.g., *Melampyrum* spp.) which could encourage the movement of pollinators between meadows. Future work could look more closely at these areas and survey visitation compared with dense forest edges with little vegetation. Bowers' (1985) study site also involved meadows divided by dense forest in subalpine meadow in Utah. He also found very little crossing by bumble bees between meadows and attributed it in part to a dearth of resources in the understory of forests, and that the economics of flying to far away meadows were probably too high. The same could apply in Norway where older stands of forest are sparse with basically no subcanopy. The Scout and Svervelli meadows were divided by a section of young, not very dense regenerating birch scrub (Figure 3.1). It may be significant that four of the five crossings by bumble bees between meadows were between these two meadows. It could also be an artefact resulting from the fact that more bees were marked in Svervelli and Scout meadows (41% of all bees were marked in Svervelli, 34% in Scout compared with 25% in the Barn meadow) and that they were bigger with more flower resources, so that the probabilities of reobservation were greater. The Barn meadow was much smaller and had limited pollen and nectar sources and was separated by much thicker forest than were the others (Plate 3.1). Plowright & Galen (1985) found that landmarks and boundaries on a smale scale appeared to influence bumble bee movement, in that they tended to move more rapidly through uniform arrays. They recognised that their study was at a small scale, and suggested that more studies should be carried out in larger structurally-heterogeneous areas with landmarks and boundaries because these may restrict bee movement. Gary *et al.* (1977) found that honey bees dispersed widely over onion fields and tended to concentrate efforts near boundaries. In Chapter 4 of this thesis, work in a lucerne seed crop showed that bumble bees also dispersed widely. This could be related to the uniform architecture of the crop resource and its surroundings and that bees find it difficult to orientate in homogeneous landscapes.

Potential influence of social caste and species on mobility

Of the five bumble bees recorded crossing meadows, three were male caste including two "cuckoo bumble bees" (*Psithyrus* spp.) - "inquiline bumble bees which live at the expense of their *Bombus* hosts" (Alford 1975). Neither cuckoo bees nor true bumble bee males collect pollen and nectar for nests (Prys-Jones & Corbet 1991) and therefore are not regarded as central place-foragers. Once males leave the nest they do not usually return. However, male caste *Bombus* and *Psithyrus* spp. could be valuable for pollen cross-over between isolated plant populations if they travel more widely than the worker caste. Perhaps it is innate for male *Bombus* to travel from the normal foraging area of their own nest to increase the genetic stability of their own populations. Species may influence mobility of bumble bees which have different habitat requirement; for example Banaszak (1983) in a survey of bumble bee habitats found that *B. terrestris* and *B. lapidarius* preferred more open habitats. Kwak *et al.* (1989) reported that *B. pratorum* and *B. jonellus* (Kirby) were more constant to a particular meadow than was *B. pascuorum.* This could have implications for plants visited by those species in increasingly closed-in meadows. These were the species in this work to cross between meadows. More work is needed on different species' habitat requirements and the relative responsibility of each for certain plant species.

Aspects for future research and implications of this work

Experimental testing of the effect of vegetation boundaries on bumble bee movement would be useful. Artificial hedge set-ups were used by Fry & Robson (1994) to test butterfly movement and by Evans (1996) for hover flies. For bumble bees this may be difficult because they are such strong fliers. Few data exist on the flight height of bumble bees but it is known to be highly variable and differs between castes (Ranta *et al.* 1981; Fussell & Corbet 1992b). Bringer 1973) recorded *B. lapidarius* flying at 17 m, but *B. hortorum* and *B. hypnorum* (L.) were not recorded above 1.5 m.

Pollen analysis of corbiculae loads of bumble bees or by dismantling nests of known locations at the end of the season in different parts of the meadow would be an alternative approach to finding out where bees have foraged. A marker plant such as *Phacelia tanacetifolia* with distinctive pollen grains that does not exist in the meadows could be used planted in known

locations, for example in meadow clearings on either side of dense forest boundaries. Methods of tracking bees using radar are currently under development based on a harmonic generating tag (Hornsby 1996). If this technology can be made powerful enough to track individuals over large areas it will provide a breakthrough for studies of insect movement at the landscape scale. Detailed measurement of pollen flow and resulting plant fitness between plant populations in different meadows as a result of bumble bee vectors should be a profitable area for future research. Suitable techniques for measuring whether plants actually are suffering from pollination-limitation are outlined in Kearns & Inouye (1993) and should be a good starting point for more focused studies of floral biology.

Conservation dilemmas

If pollination is proven to be a limiting factor to plant population success, and is linked with fragmentation effects, this research has vital relevance for the development of management programmes to preserve biological diversity in traditional hay meadows. These meadows which were man-created may need to be fully man-maintained to preserve them. Areas may be set aside as "living-operating museums" under traditional management techniques. However, if they are forced to be maintained by minimal input depending on government policy, then the key processes need to be understood in order to manipulate them. The opinion of Jennersten *et al.* (1992) was that re-establishment of traditional land use as a method of increasing habitat quality is unrealistic except in small reserves, and that conservationists must focus on satisfying the demands of crucial stages in the life cycles of key species. Pollination is considered a "keystone process" in maintaining biological diversity (Kevan 1991). Scrub management may be necessary to keep areas open with movement corridors to allow flow of pollinators, and slow the natural succession of forest which may cause plant species loss through altered habitat size and quality. Cutting hay crops only at certain times of the year after flowering herbs have set seed may be needed. Bumble bees prefer plants typically found in mid-successional habitats, not pioneering or climax species (Fussell & Corbet 1992a), which is the stage of succession at which these meadows could be considered to be. Perennials rather than annual flowering species for example are preferred (Osborne *et al.* 1991; Dramstad 1996a). In this work, all species on which bumble bees were marked and reobserved were perennials except the annual species *Galeopsis* sp. (Labiatae), and two semi-parasitic annuals *Melampyrum*

pratense and *Rhinanthus minor* (L.; Schrophulariaceae). This could be a function of the successional stage of the vegetation where perennials dominate, or a preference for perennials over annuals which was demonstrated for southern Norwegian bumble bee species by Dramstad (1995; 1996a). Holt *et al.* (1993) concluded from experiments on an artificially fragmented habitat that fragmentation affected the local persistence of herbaceous species, but did not markedly affect the overall course of secondary succession. Establishing the direct effects of fragmentation, as opposed to those of natural succession in the absence of management will be difficult to separate, and are effects likely to act in combination. For example, are herb populations decreasing because they are not being pollinated through isolation effects, or are they being naturally out-competed by light- and moisture-demanding grasses? There is debate over what the natural state of Scandinavia should be (Hansson 1992). Preserving man-made habitats, such as the traditional hay meadows of Norway examined in this work has added fuel to the debate.

In summary, the foraging patterns observed by bumble bees can be explained in terms of recognised bumble bee foraging strategies such as area-loyalty, traplining and majoring-minoring behaviour. Saville *et al.* (1996) suggested that in addition, forager distribution in patchy fragmented landscapes is likely to be influenced by nest location and forage preferences of different species. In his work in subalpine meadows in Utah, Bowers (1985) reported that workers appeared not to utilise flowers outside the meadow where their nests were located. Because different bumble bee species have different nesting requirements (Corbet & Fussell 1992), different meadow habitat sizes, physical structure, aspect, and shelter could have direct and indirect effects on pollination success within meadows. There may be a meadow size threshold for bumblebee inhabitation by queens in the spring (Bowers 1985), and later in the season meadow florisitics determine bumble bee distribution. Bowers (1985) concluded that both local extinctions and production of reproductives was highly correlated with meadow floristics. Possibly the "shrinking" of meadows alone could influence bumble bee occupancy and success in Norwegian meadows too. In the present work, location of the nest was not known, but this aspect is pursued in Chapter 4. Bumble bees in this work exhibited a high degree of area-loyalty, being restricted to relatively small areas within individual meadows where they were marked. Few individuals crossed between meadows. Multiple visits were

recorded by individuals to the same *Aconitum* patches, and mostly to nearest neighbour patches. However, non-specific generalist species were recorded on *Aconitum* patches rather than the specialist species expected, and reobservation numbers were low, partly as a result of an unsuccessful marking method and relatively low numbers marked initially. These patterns of movement may, theoretically, limit pollen flow between plant populations in different meadows.

Further research is needed to establish whether the patterns of bumble bee movement observed are the result of typical bumble bee-associated foraging strategies, decisions made based on resource distribution, or direct or indirect effects of physical landscape features. Further research is also needed to relate the observed patterns with plant reproductive success.

Chapter 4

Bombus terrestris movement in an intensively-managed habitat

4.1 Introduction

In contrast to the study in a semi-natural environment in Chapter 3, this study addresses bumble bee movement in a commercial seed crop. Movement is assessed specifically in relation to nest sites, rather than between forage patches as in Chapter 3.

Pollination, a limiting factor in lucerne seed production in New Zealand

The production of legume seed is a high-value industry in New Zealand. Red clover *Trifolium pratense*, white clover *T.repens* , and lucerne *M. sativa* are the main species grown. While much of the legume seed is used for pasture maintenance and renewal for the local New Zealand market, considerable export earnings are gained from the clover species. Lucerne seed yields in New Zealand are well below those achieved in other countries (Donovan 1974). It is hoped that through a specialist approach to lucerne seed production, yields in New Zealand can be greatly improved in order to reduce the current dependence on supplementary imported seed, and possibly export it in the future. One aspect of this specialist approach is to improve pollination rates (Dunbier *et al.* 1983).

Potential to increase seed yield of lucerne through improved pollination rates

New Zealand's bee diversity is poor compared with that in many overseas lucerne-growing countries. None of our native species are suitable for lucerne pollination (Donovan & Macfarlane 1984). Introduced lucerne leafcutting bees *Megachile rotundata* and alkali bees *Nomia melanderi* have had limited success in the New Zealand climate (Purves pers. comm.). In Canterbury, the main seed-cropping region of New Zealand, strong prevailing summer winds limit the activity of the introduced solitary bee species which are not very robust fliers. Honey bees, which are the main pollinators of lucerne in hotter overseas climates where flowers trip more easily, are not as effective in New Zealand's cooler conditions (Free 1993).

Short-tongued bumble bees *Bombus terrestris* have been identified as efficient pollinators of lucerne in New Zealand's climate (Gurr 1955, 1974). Their ability to thermoregulate, their robust bodies for flight in adverse conditions, their large hairy bodies to transfer lots of pollen, and their ability to "trip" legume flower mechanisms (see Section 2.2.2), which other bees learn to avoid, are key assets for lucerne pollination. However, their populations are currently too low and fluctuate widely between seasons, and they are not yet fully manageable in colonies on a large scale where and when needed (Donovan & Macfarlane 1984). Canterbury is a very intensive cropping region. Species and location of crops grown change every year, providing an unstable habitat for continuous forage throughout the year offering few areas of undisturbed land which bumble bees need for nesting.

The relevance of spatial information to the management of bumble bees in legume crops

Spatial information on colony foraging areas will be essential in the management of bumble bee nests in the field, whether the bees are obtained commercially, are occupied boxes which have been placed in the field, or are naturally-occurring nests (see Section 2.3.2). Information on "hot" and "cold" spots of pollination may exist in crops and these could be remedied by modifying placement of nests and nest stocking rate. Knowledge of the distance and direction bees fly is also valuable in devising isolation distances between hybrid seed crops (George 1985).

Limitations of previous studies on bumble bee movement from the nest in relation to crops

There have been few specific attempts to assess bumble bee foraging in relation to the nest site. Several anecdotal reports suggest that bumble bees generally forage within a few hundred metres of their nest (Alford 1975; Macfarlane *et al.* 1994; Corbet *et al.* 1994; Dramstad 1996b). Some individuals have been observed some kilometres from their nest (Free & Butler 1959; Banaszak 1983). Recent work (Saville 1993; Dramstad 1996a; Dramstad 1996b) assessed movement of bumble bees in relation to their nests in cereal cropping areas interspersed with semi-natural habitat remnant islands. Their work showed that bumble bees can travel far from their nests, and do not necessarily forage close to them.

The behaviour of bumble bees from nests placed near rich resources such as horticultural and agricultural crops is poorly understood. Conclusions made by different authors conflict as to whether or not bumble bees forage in the crop adjacent to their nests. In many of the following cases, results are confounded by low sample number, non-recognition of nest bees from bees in the area already, and no reports of reobservation rate to indicate how representative the data obtained was. Studies are also difficult to compare because of different sampling intensity and different sampling designs.

Case studies

Two examples in which bumble bees nests were introduced to field crops are that by Macfarlane *et al.* (1994) for cranberry pollination and by Whidden (1996) for lowbush blueberry pollination (both *Vaccinium* spp.; Ericaceae). In a glasshouse situation bumble bees are captive and will readily forage on the crop (van den Eijende 1992). In the field, a key question is how specific they will be to the target crop. Whidden (1996) concluded through using pollen analysis techniques, that in pollen loads returned by three-quarters of foragers, crop pollen constituted greater than 90% of the load. However, Whidden's sample number was very small (52 bees) and from two days only. Macfarlane *et al.* (1994) also concluded that bumble bees from nests placed in a cranberry bog limited their foraging to the crop. Macfarlane *et al.* concluded that foraging decreased 140-160 m from the hive, and that foraging area was mainly limited to within 30-60 m of it. There was no reobservation data presented, replication was low, and it was not known if the bees present were from the nests placed there, or were there previously.

The leptokurtic distribution curve of flight distances (see definition in Section 2.4.2) presented by Macfarlane *et al.* (1994) showed that most bees foraged within 50 m of the nest, but this is likely to be an artefact of the transect sampling method used. As linear distance increases from the marking point, the area of the circle (used to estimate foraging area from the colony) based on the radius of that length, increases disproportionately. This means that the probability of reobservation with increasing distance also decreases disproportionately. The resulting leptokurtic curve therefore peaks at short flight distances, then steeply declines with increasing flight distance.

Red clover crops have received the most attention in these studies. Free & Butler (1959) marked bumble bees from an artificially reared nest placed in a 1.2 ha red clover crop, and relocated bees mainly close to the nest, but their reobservation rate was also not reported. Braun *et al.* (1956) placed nests near large 30 ha and 90 ha red clover fields (presumably around the outside). No bees were found in the centres, which was attributed to their foraging close to their nests. This was not confirmed, however. It was not known where most of them foraged, or if they foraged in the crop at all. Butler (1951) concluded that bumble bees foraged no further than 18.3 m from their nest in a crop of red clover. Methods were not clearly explained. Information on trial design, reobservation rate, duration of the study, and observation effort was not reported, giving Butler's measurement no context. Macfarlane *et al.* (1990) recommended a stocking rate of 6 colonies per hectare for pollination of red clover in New Zealand. They commented that proper colony placement is essential because of the limited foraging range of bumble bees. However, this was based on a model built largely on temporal data, which did not take spatial aspects of foraging into account, and foraging range was not measured.

Theoretically, bumble bees should forage in a rich resource (such as a crop) when their nests are placed close to it in order to maximise energy gains. Heinrich (1976) argued that "as long as food is locally abundant, it is probable that most bumble bees forage close to the nest". Baker (1992) and Hågstrom (1992) cited in Saville (1993), moved garden-occupied bumble bee boxes of *B. pascuorum* to a large patch of *Echium vulgare* (L.) and marked over 100 bees from those nests. Few marked bees were found more than 10 m from the nest, and none any further than 30 m. Saville (1993) concluded that at least "in the presence of such an abundance of suitable forage close to the nest it seems that some bees do forage at short distances from the nest".

However, phenomena of "mysteriously disappearing bumble bees" from nests placed adjacent to rich resources have also been reported. Saville (1993) recorded very low reobservation rates for *B. pratorum*, *B. hortorum*, *B. ruderarius,* and *B. pascuorum* in nest experiments. Dramstad (1996b) placed several nests next to a strip of *Phacelia tanacetifolia* several hundred metres long. Bees were marked and nest traffic recorded. Few of the nest bees visited the strip.

After nests had been moved ca.300 m away from the strip, bumble bees increased their usage of it. Dramstad concluded that bumble bees may not necessarily "prefer" to forage close to their nests. In fact, spacing out could be an innate mechanism for avoiding nest predation. Hobbs *et al.* (1961) placed artificial colonies of *B. terrestris* and *B. borealis* next to plots of clover where there were also naturally-occurring colonies. Workers from the natural nests visited clover plots, but bumble bees from artificial colonies did not, even though there was a supposedly rich food source only metres away.

The aim of this chapter was to determine the foraging patterns of bumble bees from several nests placed adjacent to a lucerne seed crop using a replicated and more thorough approach than past studies in commercial crops. It was assumed that bumble bees would find the resource "attractive" and that most of them would forage within it.

Specific aims of this chapter were:

- to develop an efficient system to mark automatically bumble bee foragers leaving nests
- to reobserve the entire crop area with equal effort for every part, in order to avoid results being influenced by a common mathematical sampling artefact- the leptokurtic distribution of flight distances, encountered by previous authors (see Section 2.4.2.)
- to estimate the crop-specificity of bumble bees from nests placed beside the lucerne crop, by comparing time-lapse video records of nest traffic data with reobservation rates of marked nest-bumble bees in the crop.
- to use novel graphical methods to identify areas of intense bumble bee usage ("hot spots") within the crop, then to test statistically any correlations between bumble bee and flower distribution.
- to establish foraging areas of bumble bee colonies
- to correlate spatial and temporal patterns of foraging observed, with resource abundance and distribution, microclimate data known to influence flight activity, presence of honey bees, and variation in reproductive success between nests.

4.2 Methods

Experimental field plan and bumble bee nest shelter design

Eight commercially reared colonies of *Bombus terrestris* were obtained from Pacific Wide (NZ) Ltd. (a Christchurch-based company). These were placed along two sides of a 16 m x 150 m lucerne seed crop, allowing even spacing between nests in early February 1994 (Appendix 4.1). Bee nests were placed in the field 10 days prior to marking to enable them to adjust to the field environment. Colonies were not fed sugar syrup or pollen supplements for a week before or during the study period. Stocking rates for bumble bees in lucerne crops have not been established, but for red clover a rate of approximately 6 colonies per hectare was recommended by Macfarlane *et al.* (1990). Eight colonies were chosen in this work to provide a form of replication, to achieve high reobservation rates in the small (ca. 0.3 ha) lucerne crop, and to observe any variations in activity between nests. The artfificially-reared colonies were originally intended for glasshouse use, and came housed in a light plastic box, inside a cardboard carton. To go into the field colonies required extra protection against light, heat, wind and condensation build-up. A square wooden honey bee "super" frame was placed over each plastic colony box, and the outer cardboard casing discarded (Plate 4.1). Shelters were further protected with reflective screens, made of 0.5 m^2 polythene sheets, painted silver and held at an angle using four wooden stakes (Plate 4.1). These were oriented to shelter colonies from the prevailing north sector winds, and the sun in the hottest part of the day.

Marking system to enable recognition of bumble bees from different nests

A method of dusting honey bees with fluorescein and rhodamine compounds by forcing them to brush past velveteen strips as they left the hive, was developed by Smith & Townsend (1951). Foragers though, had to be killed for colour identification under ultra-violet light. Instead, for this work, a non-destructive technique was developed. This was based on a marking-dispenser system designed for honey bees by Boylan-Pett *et al.* (1991) using "Day-Glo" powders (Appendix 4.2) which are visible in daylight (Plate 4.2). A non-destructive method is important because bumble bee nests contain small numbers of workers (usually 50-200), compared to honey bee colonies which can reach tens of thousands. Bumble bees were automatically marked as they left the nest.

A marker tunnel was assembled using a plastic 1.5 l plastic bottle and painted black. The end of the bottle was cut off. A plastic screw-top jar of the same diameter as the bottle was fitted to the inside of the bottle's wide end. A hole was cut in the jar's lid, to match the exit-hole of the shelter box. This lid was tacked to the side of the wooden shelter, matching the exit holes in both. This allowed the marker tunnel to be screwed on and off to recharge with fluorescent powder and adjust parts if necessary, without disturbing the bees. The bottle´s neck rested on an alighting pad (Plate 4.1). Inside the horizontal bottle, a layer of corrugated cardboard was dusted with one of eight different-coloured fluorescent powders (Plate 4.2; Appendix 4.2). A cosmetics brush dowsed with powder, protruded vertically into the bottle behind its neck . In this way, bees leaving the nest and returning to it were marked on their ventral and dorsal surfaces. The bottle was recharged with powder at least one hour prior to each reobservation session in the crop. To recharge tunnels for marking, two teaspoons (approximately 10 g) of powder was put into each bottle and shaken, before screwing it back on to the base end attached to the shelter. This provided reliable continuous marking of bumble bees entering and leaving the nest for at least two and a half hours.

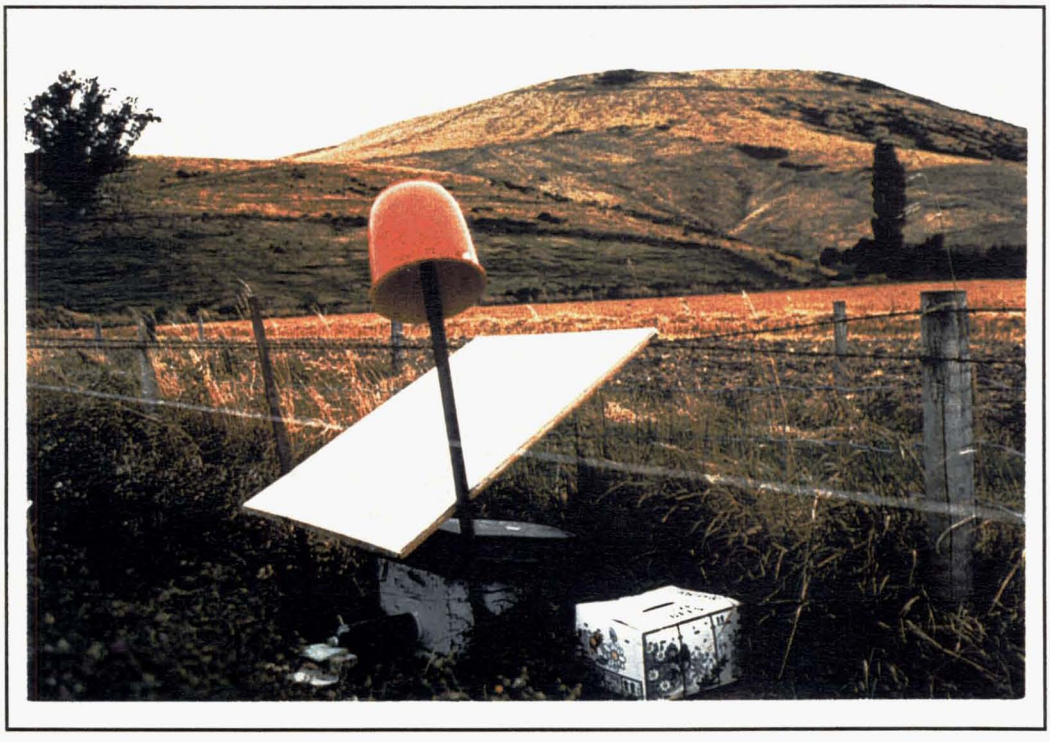


Plate 4.1. Bumble bee nest shelter showing fluorescent-powder marking tunnel attached, and bucket to guide orientation of returning foragers.

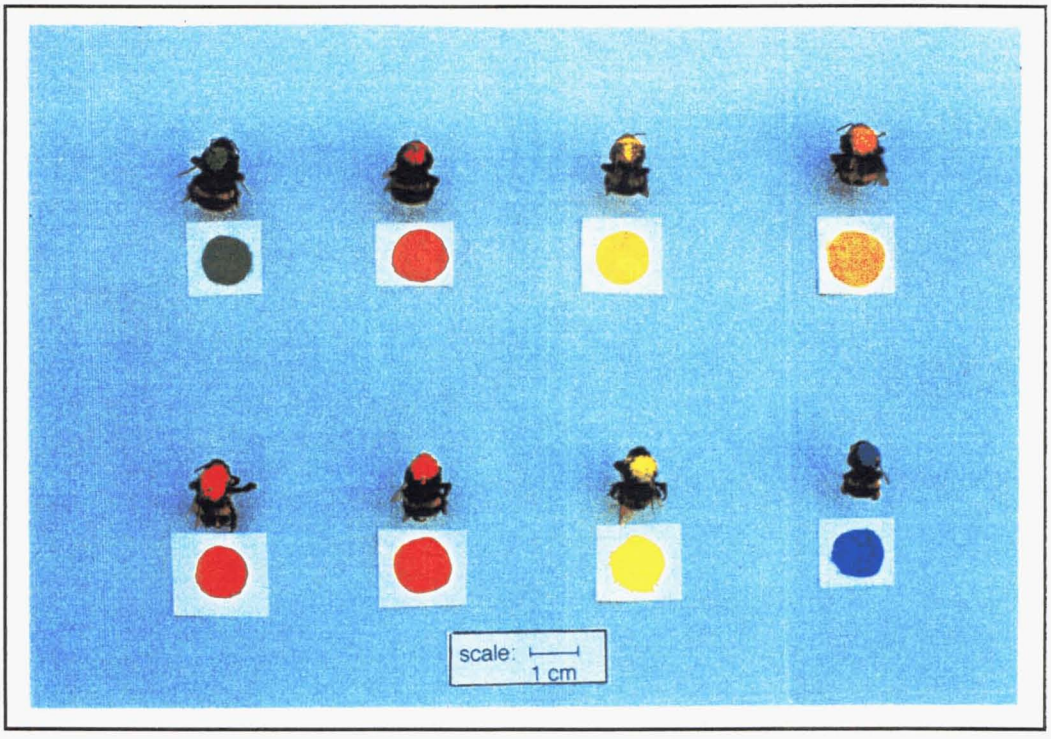


Plate 4.2. Fluorescent powder colours used in marking tunnels attached to bumble bee nests.

Reobservation strategy for marked bumble bees

The field was divided into 4 m x 4 m cells (Plate 4.3). Transect walks, as recommended by Dylewska *et al.* (1970) were performed along adjacent cell edges, observing 2 m to either side of the line to record bumble bee foraging in the whole grid and hence whole crop. Each crop reobservation took 1h 15 min. (30 s per cell). Data were recorded as points on a scale map of the crop and later, distances were manually measured from nest locations. The error using this method was calculated to be approximately ± 0.5 m. Vector distance and direction data were established from known nest positions on the field map. Bumble bees were counted, mapped and identified during transect walks. Bumble bee colour, caste, species and activity was recorded.

There were 19 reobservation sessions on 15 days over a five-week period from 4 February to 8 March. There were three dates which had more than one observation; 4 February (2), 9 February (2), and 4 March (3). Observation sessions in this experiment were based on the main activity periods of bumble bee activity. Several authors have reported a trough in bumble bee activity around mid-day due to unfavourably high temperatures (reviewed in Plowright & Laverty 1984; Alford 1975). Donovan (pers. comm.) and Holmes (1961) stated that the

physical presence of honey bees alone, in the crop may reduce bumble bee activity. A bumble bee's thermoregulatory ability (see Section 2.2.2) permits it to forage early morning and late evening when there is little competition from other flower visiting insects. As well as avoiding overheating by staying in the nest at this time, nectar is often scarce in the middle of the day (Prys-Jones & Corbet 1991). Preliminary manually-recorded nest traffic data confirmed the main period of nest traffic in this lucerne study to be in the late afternoon and early evening, peaking between 1500 and 1800 hours. This was confirmed later in the season from time-lapse video recordings (see Section 4.3). Observations were carried out whenever possible between these times. Nests became active each morning between 0830 and 0845 hours on most days.

Honey bee phenology and density

Honey bee numbers were counted in 11 sessions from 15 February onward, simultaneously with bumble bee counts. Total honey bees in every crop square were recorded while looking for marked bumble bees. To assess any competition effects, total honey bees over all sessions were correlated with total bumble bee numbers for all crop square data combined.

Recording nest traffic in order to calculate reobservation rate of marked bees in the crop

A time-lapse video cassette recorder (JVC model SR-L900E) linked to a black and white camera (JVC model TK-S240) with a 50 ml macro lens was used to record all bees entering and leaving one nest (the pink nest) over one day. Two 12 V batteries wired in parallel were sufficient to power record for 12 hours on slow-play speed using a standard 3 hour length tape. Images were taken every four seconds. The camera, mounted on a tripod was focused to the nest entrance. Traffic data was later transcribed while watching the tape replayed through a television monitor and sorted into 30 minute intervals. To calculate number of bees out foraging for each interval, in-coming bees were subtracted from out-going bees to achieve a series of cumulative totals throughout the day. To estimate reobservation rates, data from the one video recorded nest was extrapolated to estimate total number of bumble bee workers out of all nests for each observation session. This was then matched to the number of marked bees actually observed in the crop in each session. It was assumed that all nests had equal numbers of workers.

Flower density and distribution

Peak flowering was predicted by the grower to be in mid to late February, but it is variable by several weeks between years (McCartney pers. comm.). Flower abundance and distribution were scored on three dates, each two weeks apart over the experimental period; 10 February, 24 February and 7 March. Each crop cell (16 m^2) on each date, was assigned a relative score of flower density between "one" (relatively low density) to "five" (relatively high density). These scores were correlated to real flower densities as follows; For each of the three dates, a typical representative from each score group was sampled using a 1 m^2 quadrat. Number of inflorescences and flowers per inflorescence were counted, then multiplied to produce total flowers/m^2. The travelling mean method (see Wratten & Fry 1980) indicated that five samples per crop cell produced ± 10% accuracy of results. A random number table was used to generate co-ordinates for unbiased placement of each quadrat within the crop cells sampled. Results for the three scoring dates were pooled to produce mean flower density in each crop cell over the season. These means were correlated with total bumble bee numbers in each crop cell, and were the figures used to construct the colour plots of flower distribution (Figure 4.5, Section 4.3).

Graphical techniques to illustrate the spatial distribution of bumble bees and flowers

In order to visualise potential "hot and cold" areas of pollination by bumble bees within the crop, colour plots and three-dimensional mesh plots of bumble bee and flower distribution were produced using the programmes Sigma Plot and Transform for the Apple Macintosh computer by Dr. G. Fry, then modified using Canvas, also an Apple Macintosh programme.

Gauging variation in reproductive success between nests

Total number of bumble bees and the relative numbers of each caste produced (queen, worker and male) were counted by dismantling each nest at the end of the summer when nests had died. Hatched and unhatched wax pupal cells were sorted for counting into three caste groups according to cell dimensions. The dimensions of a random sample of 30 cells for each caste category over all nests was measured to confirm whether cell-counting is an accurate way to determine numbers of workers produced. Cells containing mature bees which had died before hatching were removed, and correlated with the length and diameter of their cell of origin. Definitions of cell length and width categories respectively were as follows: "large" (queens and

males); 15-22 mm x 12-14 mm, "medium" (most workers); 10-14 mm x 8-11 mm, "small" (nest bees which do not usually forage); 8-10 mm x 6-8 mm. At the time of purchase from the commercial rearer, all nests contained approximately 40 workers in good health (van den Ende pers. comm.). Therefore, any large variations in cell numbers at the end of the summer could be expected to be a result of field conditions.

Microclimate measurements

Microclimate factors influence the pollination process directly (on the insects) and indirectly through pollen and nectar production (Corbet 1990). Particularly important factors influencing flight and foraging activity of bumble bees and honey bees are temperature (Corbet *et al.* 1993; Wratt 1968), light intensity (Løken 1954), ultra violet radiation (Barth 1991), and wind (Grimm 1988; Woodell 1978). These microclimate details were recorded at the start, middle and end of each observation session to monitor any major fluctuations which might explain unusual bee behaviour. Manual equipment was positioned at crop height, 30 cm above ground. A standard manual wet-dry bulb thermometer was used for temperature, for ultra violet light levels a sensor (model Delta-T UV) linked to a multimeter reader, a black and white solarimeter (Eppley model 8-48) for visible and infra red radiation intensity, with attached multimeter to read mv output, and a rotating vane anemometer (model DVA 6000T) for windspeed. Wind direction was also noted.

On the day that diurnal nest activity was measured, "Black-globe" temperature was recorded. The aim of a "Black-globe" is to imitate the temperature the insect experiences (Corbet 1990). A common method is to use a ball of Blu-tack, coloured black and attached to a thermocouple. Bumble bee body size and pile covering is likely to effect heat absorption, so in this work a dead bumble bee worker's body was filled with silicon and the thermocouple placed inside to achieve a more accurate representation.The thermocouple was linked to a Tiny-talk® data logger which automatically recorded temperature at 20 minute intervals over the entire day.

Plate 4.3. The experimental lucerne crop before flowering, surrounded by cereal fields in mid Canterbury, New Zealand. White grid markers are visible in the crop.

4.3 Results

Summary of reobservation data

A total of 287 bumble bees was seen in the crop over all 19 reobservation sessions. Of those, 19 (7%) were *B. hortorum* and 268 (93%) were *B. terrestris*. Of the *B. terrestris* 197 (74%) were marked and 71 (26%) unmarked. Most (65 %) of bumble bees recorded as "unmarked" definitely had no colour and the remaining 35% moved away too quickly to recognise whether they were marked or not. Very few *B. terrestris* were observed in the crop before nests were introduced. *B. terrestris* recorded as "unmarked" could have been non-nest bees or nest bees which had lost their colour mark. Workers constituted the largest proportion (90%) of all bumble bees observed. Of the 197 marked bumble bees observed, 97% were workers, 1% queens and 2% males. Proportions of numbers of bumble bees observed of each marking colour are shown in Figure 4.1. Most *B. terrestris* seen on lucerne were tripping it and collecting pollen. Most bumble bees also tripped flowers as they visited for nectar. Only a few very small workers managed to rob nectar from the side of flowers without tripping them.

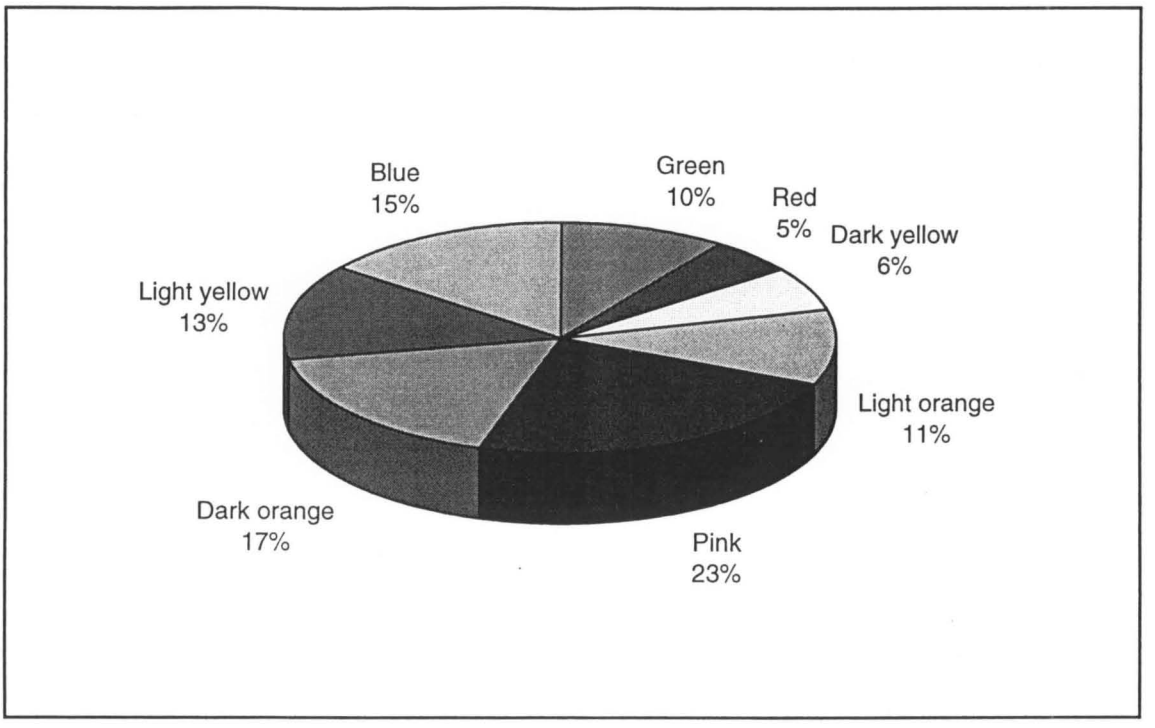


Figure 4.1. Proportional breakdown of all colour-marked bumble bees observed in a lucerne crop (n=197).

Spatial distribution of bumble bees within the crop

Prior to the lucerne reaching full flowering in mid February, few marked bumble bees were observed in the crop. Anecdotal records, not part of the experimental reobservation design, showed that some bumble bees foraged up to 750 m from their nests.

Different maximum flight distances resulted from bees from each nest (Table 4.1). A frequency distribution (Figure 4.2) of pooled results for all bees reobserved within the crop shows that most bumble bees travelled relatively short distances to forage within the crop. On average, 81.3% of bees foraged within 50 m of the nest (Table 4.1). Approximately 56 % of bumble bees foraged within 20 m of their nest. The highest frequency of flight distances was in the 15 to 19 m class. The modal flight distance was 17.5 m within the crop. Fewer than 3% of bees travelled further than 100 m within the crop (Figure 4.2).

A three-dimensional mesh graph (Figure 4.3, graph a.) and a colour density plot (Figure 4.4) indicates that there were concentrated areas of bumble bee density down the west side of the field and in the top left corner facing north compared with the eastern and southern edges, and crop centre. The variance/mean ratio index of dispersion (2.0), indicated a significantly

clumped distribution of bees ($P < 0.001$). Figures 4.3 (graph b.) and 4.5 respectively show a mesh graph and a colour plot representing flower distribution in the crop. The spread of density appears more even, with fewer and less marked peaks compared to the bumble bee plots. Figure 4.4 shows bumble bee distribution in colour, with flower densities superimposed as contour lines, and Figure 4.5 shows the reverse; flower distribution with bumble bee contour lines superimposed. Some areas of high bumble bee and flower densities appear to correspond with each other.

Table 4.1. Flight distances for bumble bees from eight nests bordering a flowering lucerne crop

Nest	1 Green	2 Red	3 Dk. yellow	4 L. orange	5 Pink	6 Dk. orange	7 L. yellow	8 Blue
Maximum distance travelled (m)	101.0	64.5	84.5	90.5	79.0	82.5	74.5	83.0
% bees travelling < 50 m	73.7	80.0	83.3	95.2	84.8	82.3	61.5	89.6
Total number of bees re-observed for each nest	19	10	12	21	46	34	26	29

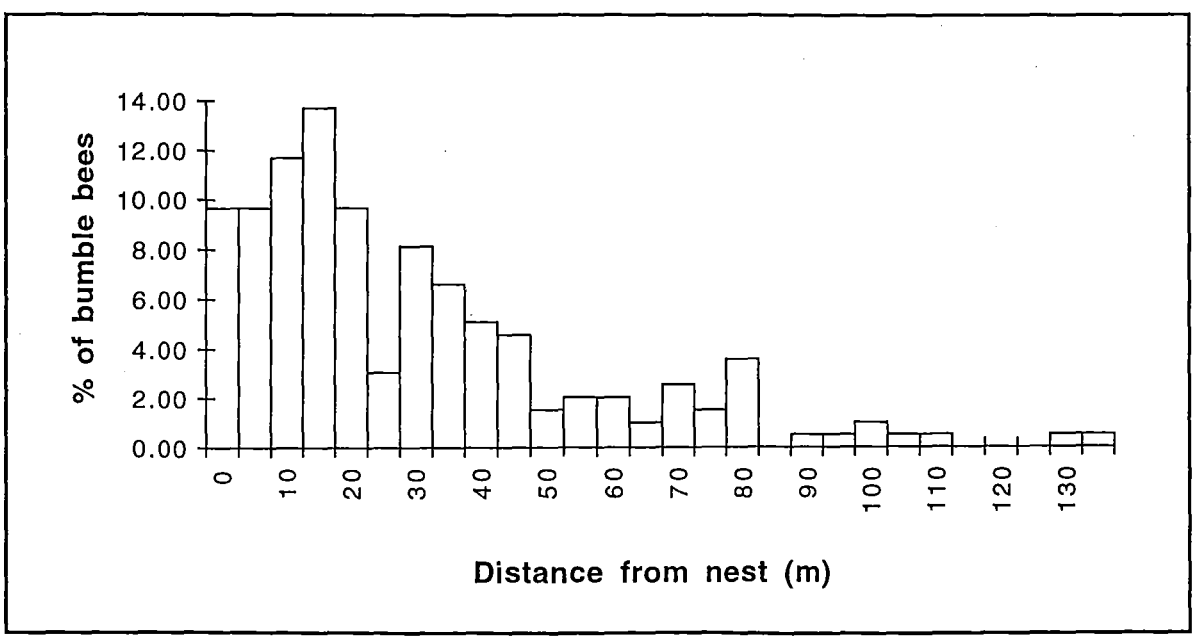


Figure 4.2. Frequency distribution of flight distances for bumble bees observed within a flowering lucerne crop

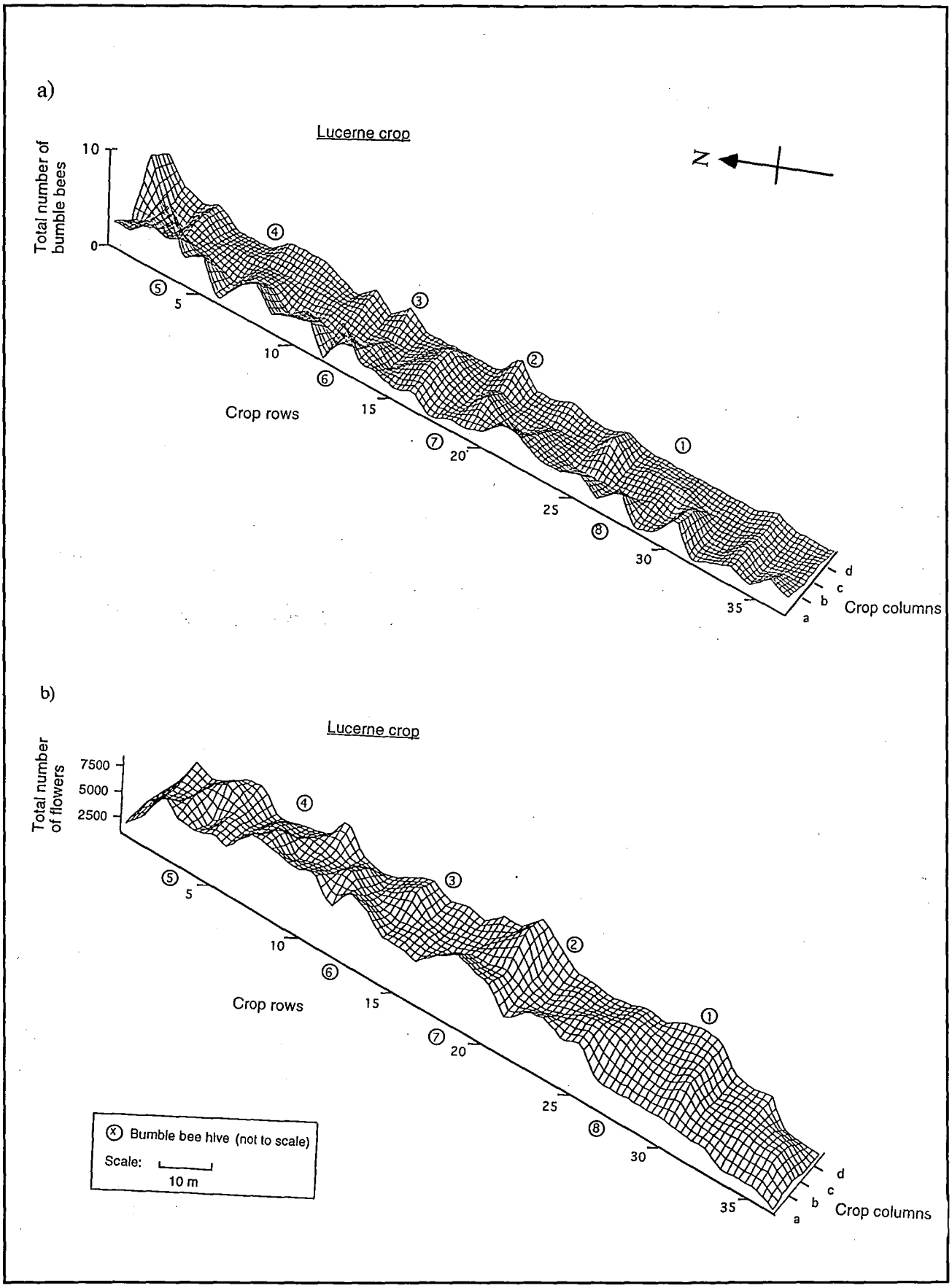


Figure 4.3. Three-dimensional mesh plots representing a) bumble bee distribution and b) flower distribution in a lucerne crop, when eight commercially-reared nests were placed on the crop perimeter. Peaks represent highest concentrations of bees and flowers. Nest colours: 1. Green, 2. Red, 3. Dark yellow, 4. Light orange, 5. Pink, 6. Dark orange, 7. Light yellow, 8. Blue.

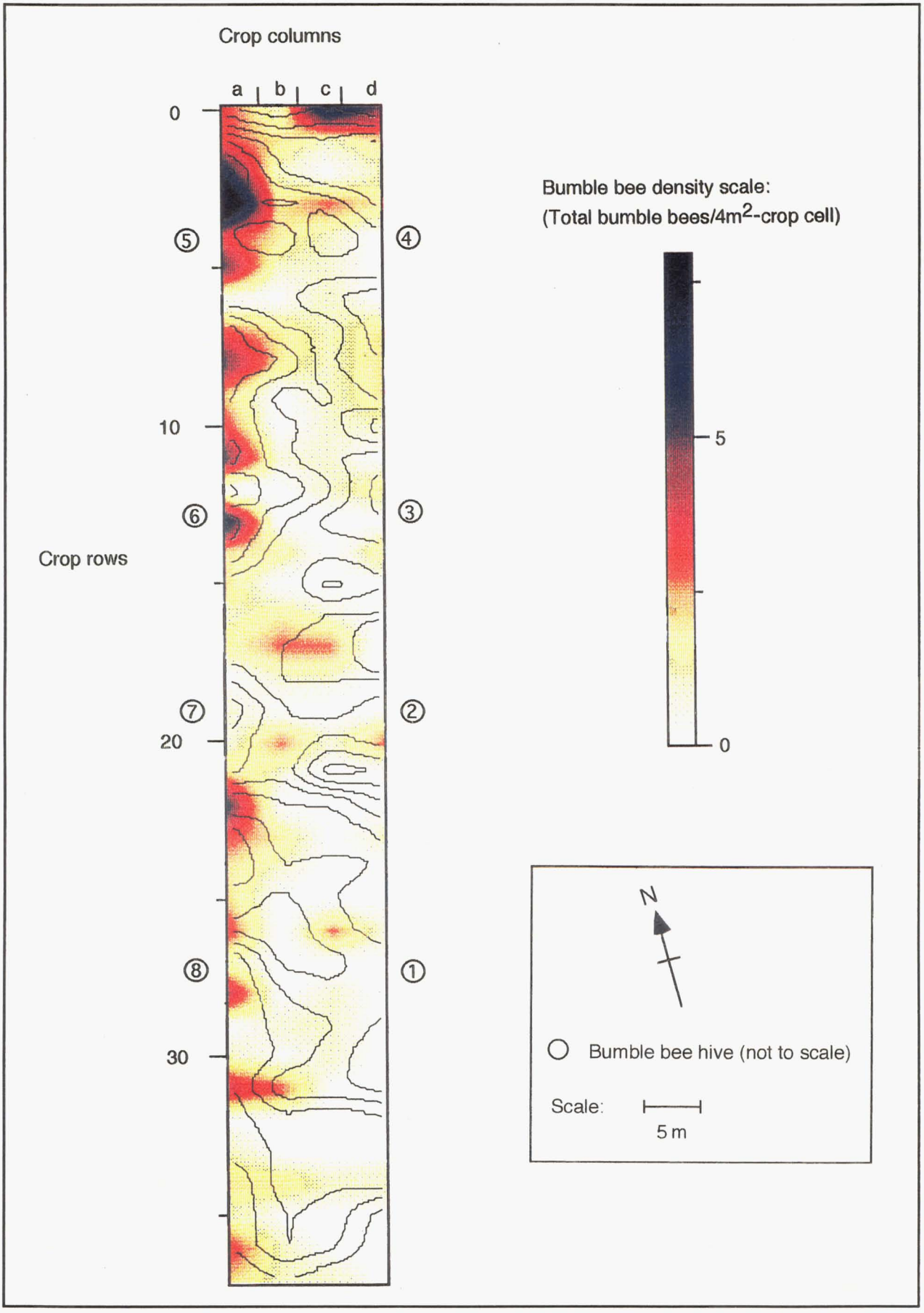


Figure 4.4. Bumble bee densities in a lucerne crop with flower densities overlaid as contour lines. Each progressive contour line indicates an additional tenth of the range of flower density. Figure 4.5 shows the reverse version of this plot (bumble bee contour lines superimposed on flower densities). Nest colours: 1. Green, 2. Red, 3. Dark yellow, 4. Light orange, 5. Pink, 6. Dark orange, 7. Light yellow, 8. Blue.

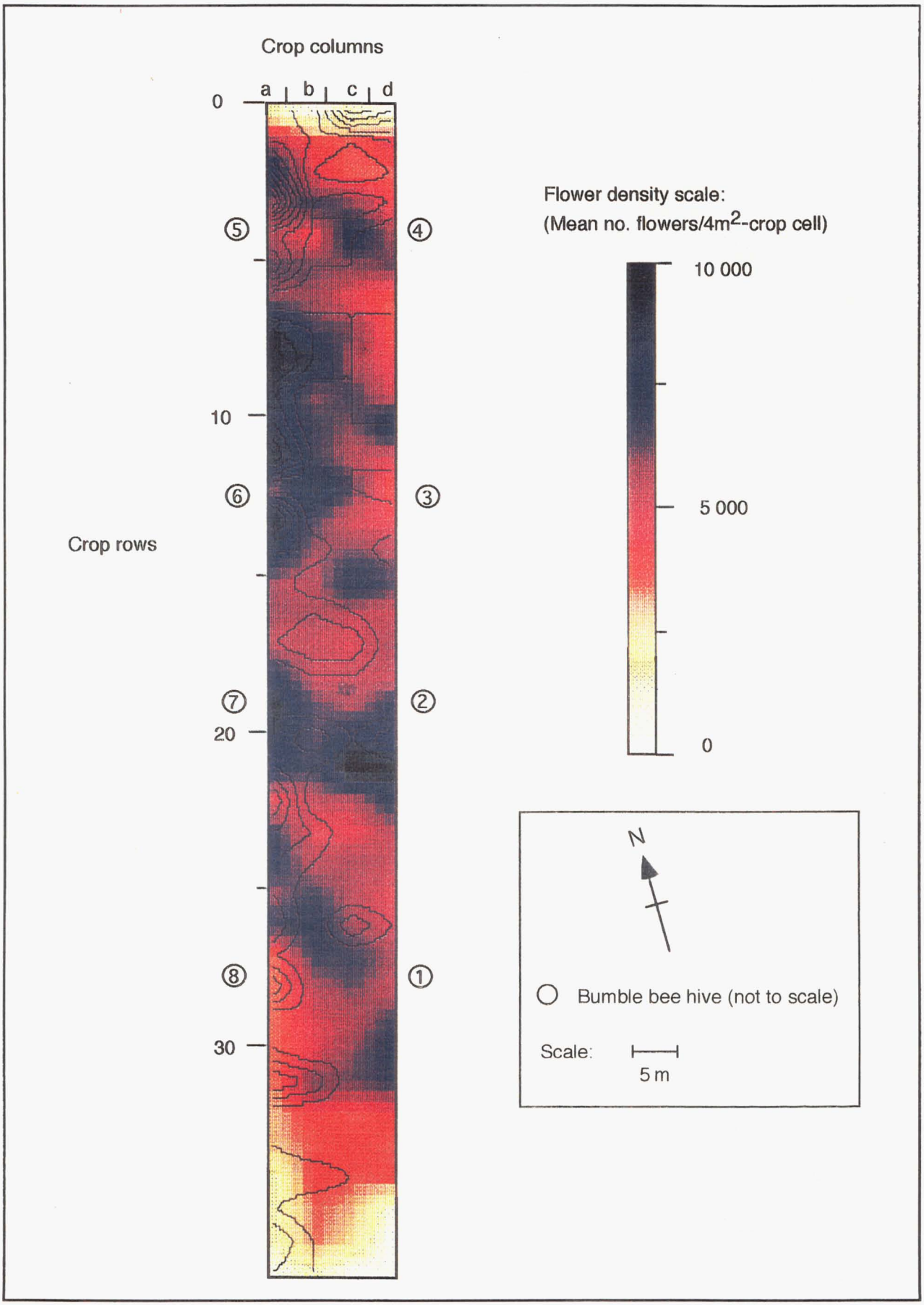


Figure 4.5. Flower densities in a lucerne crop with bumble bee densities superimposed as contour lines. Each progressive contour line indicates another tenth of the range of bumble bee density. For the reverse of this plot (flower contour lines overlaid on bumble bee densities) see Figure 4.4. Nest colours: 1. Green, 2. Red, 3. Dark yellow, 4. Light orange, 5. Pink, 6. Dark orange, 7. Light yellow, 8. Blue.

Total bumble and honey bee numbers over time, and in relation to flower production over time

There was an increase over time in bumble bee numbers but on 5, 6, and 7 March a drop in numbers occurred (Figure 4.6). These sessions were in the morning unlike the rest of observation sessions. Honey bees were at their highest (700 total) in the crop on 4 March. Figure 4.6 shows flower numbers decreasing from mid February. It is not possible to conclude that mid February was the peak as predicted by the grower. There was a weak positive correlation between mean flower density over all reobservations and total bumble bees observed over all sessions (calculated from data for each crop square). This relationship though was not statistically significant (Spearman rank; $P > 0.05$). There was a weak negative correlation between total honey bees per crop grid and total bumble bees, which was not significant (Spearman rank; $P > 0.05$).

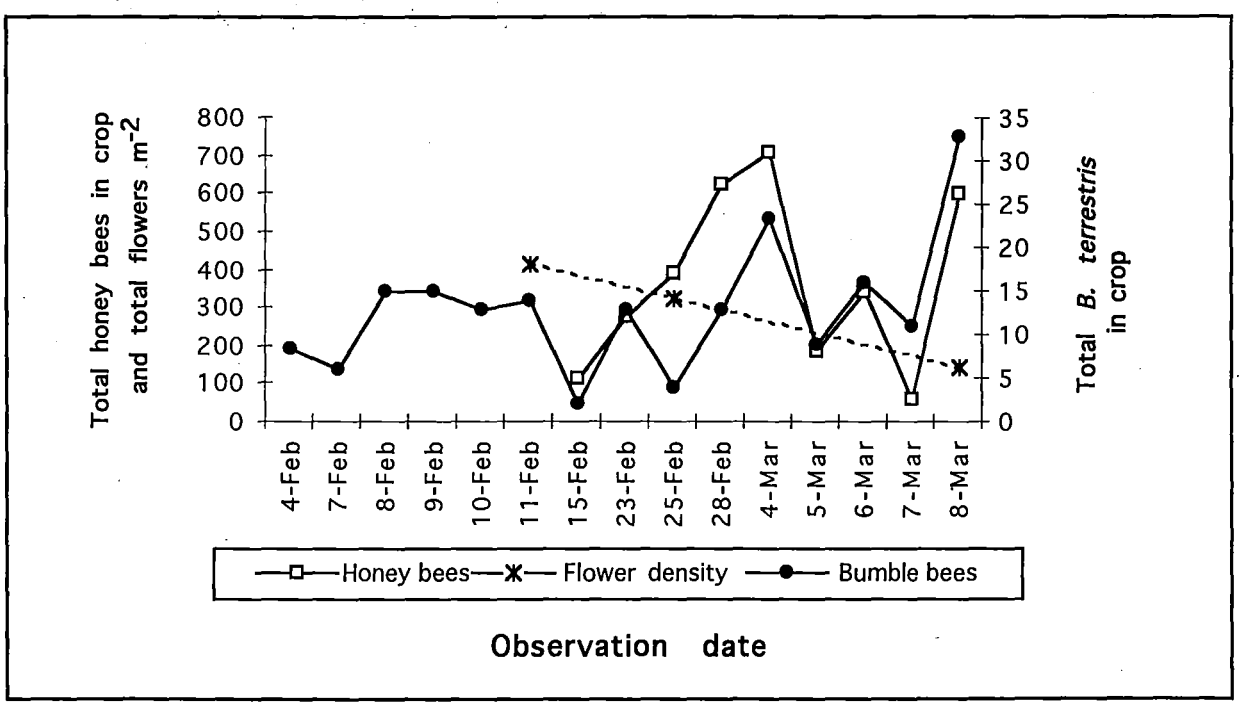


Figure 4.6. Flower, bumble bee and honey bee phenology in the lucerne crop over the five week observation period

Variability in reproductive success between nests

Height and width of cells produced for each caste were remarkably constant (Figure 4.7), indicating that the method used to estimate numbers of bumble bees in each caste produced over the season was accurate (see Section 4.1 for cell size criteria and definitions). Total number of cells produced per nest were highly variable between nests, ranging from under 200 in the green nest to over 700 in the blue nest (Figure 4.8). The proportions of male, worker and queen caste for each nest were very similar (Figure 4.8). Most cells in each nest were hatched (Figure 4.8), indicating similar phenology of all nests, and that nests were almost at the end of their cycle when the experiment finished.

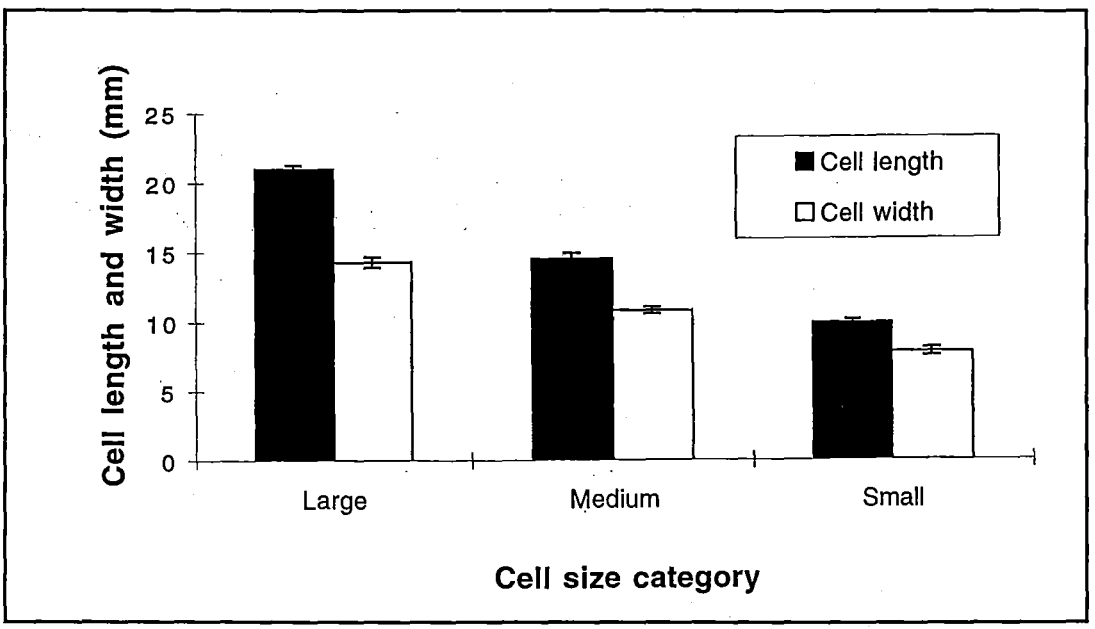


Figure 4.7. Mean bumble bee pupal cell measurements ± SE. Large = queens and males, medium = most workers, small = nest bees.

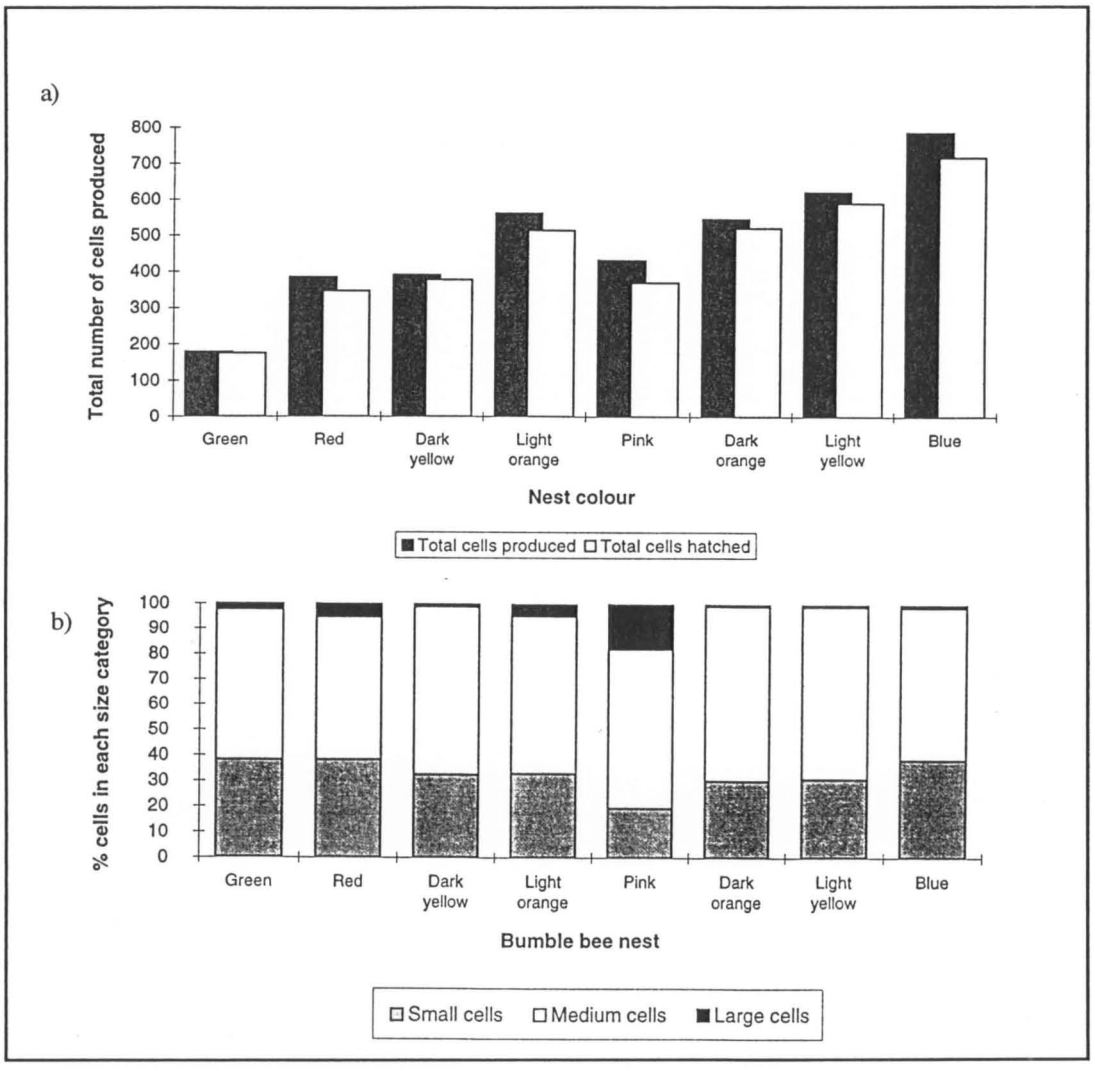


Figure 4.8. a) Bumble bee larval cell production in each nest. b) Proportions of queen, worker and male cells produced in each nest.

Nest activity level

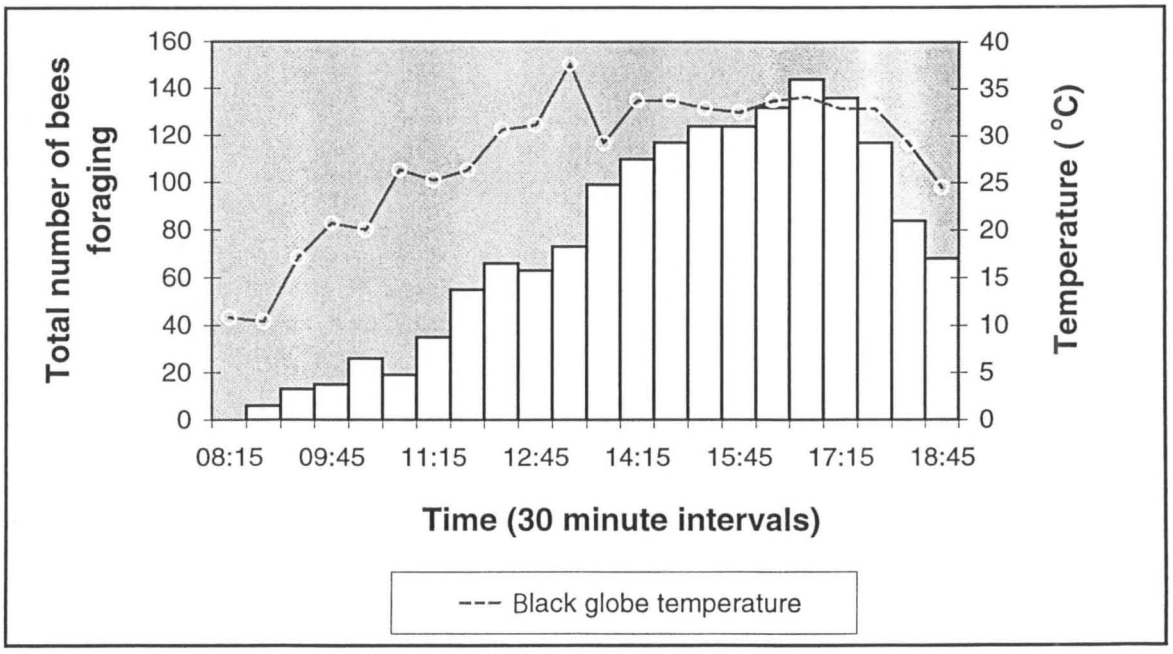


Figure 4.9. Diurnal pattern of bumble bee foraging activity in a lucerne crop based on time-lapse video recordings of one nest (pink marker) on 4 March 1994, also showing Black-globe temperatures.

Marking and reobservation efficacy

Nest entrance recordings showed the fluorescent powder to be effective for at least 2 and a half hours after application despite intensive grooming by some bees. Powder accumulates particularly well in wing and leg joints, and bumble bees can not reach an area on the top of their thorax (Plate 4.2). Reobservation rates estimated from time-lapse video information indicated that the proportion of bees observed in the crop ranged from less than 0.2% to 2.4% (see calculations in Section 4.4). In occasional transect walks outside the crop, marked bees were observed on various weed species: Californian thistle *Cirsium arvense* (L.), Purple loosestrife *Lythrum salicaria* (L.; Lythraceae), Scotch thistle *Cirsium vulgare* (Savi), and Birdsfoot-trefoil *Lotus corniculatus*. Bees returned with distinctively-coloured purple loosestrife pollen. Most foraging activity in this experiment occurred in the late afternoon, peaking at 1700 hours as shown in Figure 4.9. The rate of marked bumble bees observed in the crop of all *B. terrestris* increased rapidly from 20% on the first reobservation, then increased to 100% on the second reobservation, and averaged at 75% overall when all reobservations were combined (Figure 4.11).

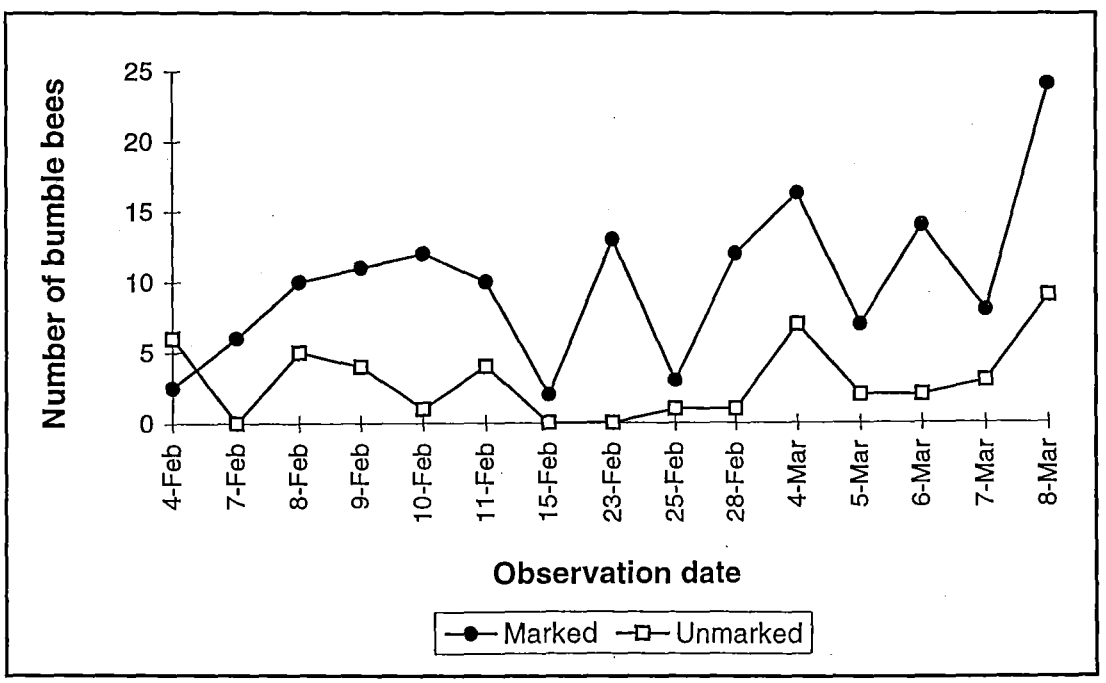


Figure 4.10. Total marked and unmarked *B. terrestris* observed on each reobservation date

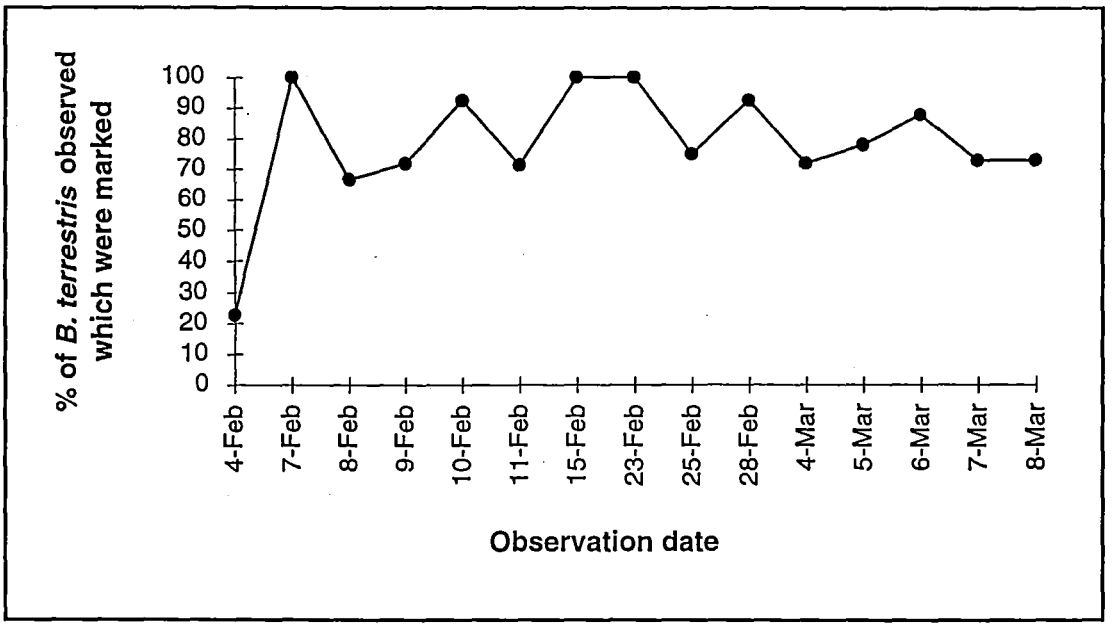


Figure 4.11. Proportion of marked *B. terrestris* out of all *B. terrestris* observed on each marking and reobservation date.

Presence of honey bees

Figure 4.6 shows the phenology of honey-bee numbers. A Spearman rank test showed a highly significant ($P < 0.001$) but weak negative correlation between total honey bee and bumble bee numbers over all observations.

Climatic factors

Microclimate details were relatively constant for all observation sessions, except for a very high temperature (> 35° C) in the 15th session (4 March) as shown in Figure 4.12. Most winds were from the north-west. Historical data recorded at the Lincoln weather station confirmed that approximately 60% of the winds in the study period were from the north sector (Cherry 1994). On the "dawn to dusk" day of video-recording, Black globe-temperature reached a peak at 1300 hours (Figure 4.9).

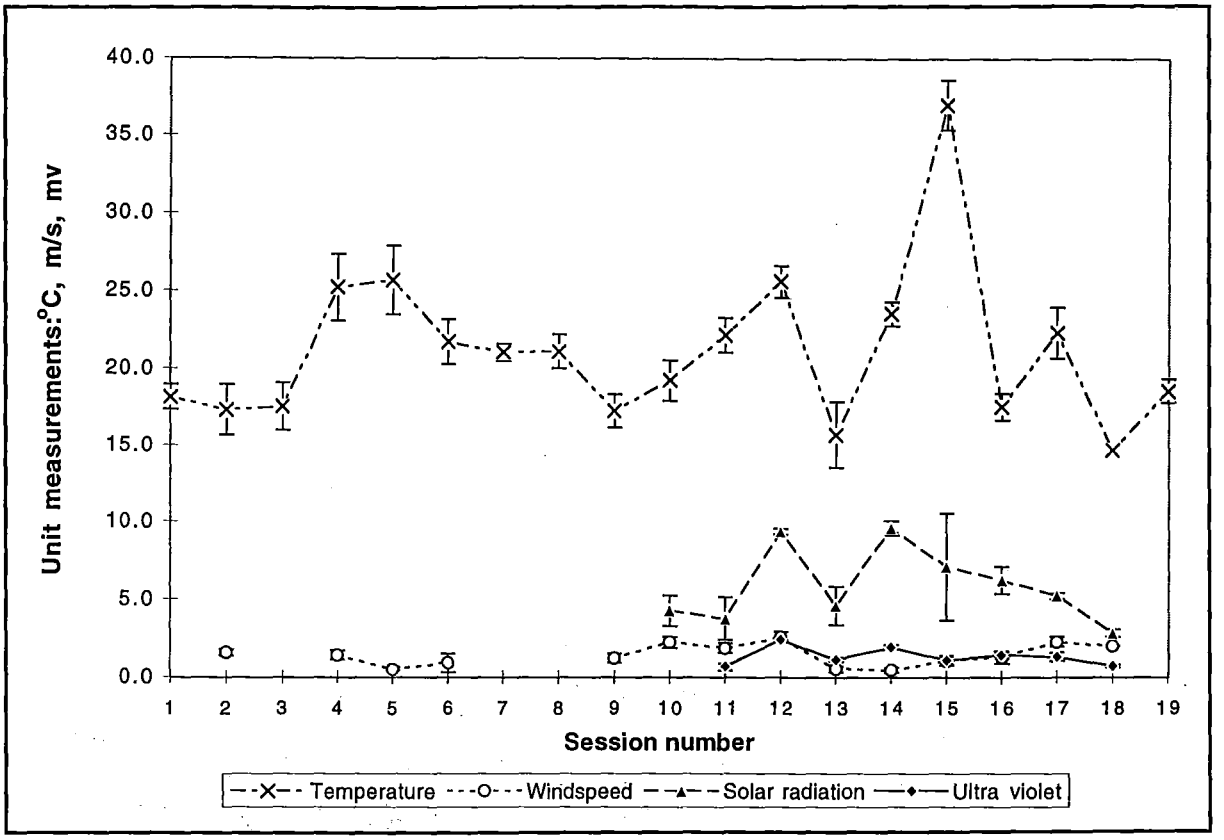


Figure 4.12. Microclimate details important in determining bee flight activity. Mean data ± 95% C.L. for each reobservation period. Temperature (°C), windspeed (m/s), solar radiation (mv), ultra violet light level (mv).

4.4 Discussion

A key finding of this work which has serious implications for the interpretation of results, is that *most* bumble bees from the introduced nests did *not* visit the crop (< 1%). That most bumble bees did not necessarily forage close to their nest supports findings by Dramstad (1996a,b). The result that bees were not specific to the lucerne crop was unexpected. Lucerne is reported to be highly attractive to bumble bees. It was assumed that the crop would provide a rich resource, and being close to nests, bees would minimise their flight distances to maximise energy profits. It was not possible therefore to fulfil the main aim of this work, which was to construct foraging areas for each nest, because it was not known where the other 99% of the bumble bees foraged.

This discussion firstly clarifies how reobservation rate calculations were made, then focuses on the spatial patterns observed for the individuals which *were* seen in the crop, and discusses possible reasons why most bumble bees foraged elsewhere.

Low reobservation rates of marked bees; calculation and caution

Calculations are as follows; time-lapse video records of the pink nest, during the peak traffic period (1500 to 1800 hours) when reobservation sessions took place, showed that an average of 128 bumble bees were foraging (Figure 4.9). Data from the seven 30-minute intervals (14:45-15:15 to 17:45-18:15) were used for that calculation (Figure 4.9). Extrapolated for 8 nests, this means that approximately 1020 bumble bees should have been foraging at this time. This calculation assumes equal numbers of workers in each nest. The pink nest was a good representative nest to record because it was in the middle of the range for number of workers cells produced (Figure 4.8). On average 10 bumble bees were seen per session in the crop. Therefore reobservation rate on average was: 10 bumble bees in the crop per session as a percentage of 1020 bumble bees foraging, i.e., 10/1020 x 100 = 0.98%. The number of marked bumble bees observed per session ranged from 2 to 24 (Figure 4.10). Therefore, reobservation rates ranged from 0.2% (2/1020 x 100%) to 2.4% (24/1020 x 100%).

This conclusion that most bumble bees foraged outside the crop area is supported by the fact that several differently-coloured pollen loads were returned to nests by foragers. Lucerne pollen is a pale beige, unlike the many yellow, brown and orange coloured pollen pellets returned. In occasional searches of the surrounding area, marked bees were seen foraging on a range of other species including, *Lotus corniculatus* ,*Cirsium arvense* , *Brassica napus* L. crop remnants, and purple loosestrife *Lythrum salicaria* up to 750 m from the crop. It was physically impossible to search the entire area surrounding the crop. A circle with 750 m radius encloses 176.7 ha.

Diurnal activity pattern of bumble bees, and effects of microclimate for all sessions

Results in Figure 4.9 clearly confirmed a peak of bumble bee activity between 1500 and 1800 hours which did not match peak daily temperature (1200-1300 hours) presented by "Black-globe" recordings. This supports literature findings that bumble bees avoid the hottest part of

the day. Although bumble bees can thermoregulate to cool themselves, it is probably energy-expensive to do so. Bumble bees can store enough food for a few days at least, in case extreme weather conditions persist (Donovan pers. comm.). Bumble bees were heard "fanning" their nests to keep cool. Several workers actually un-couple each wing pair (which are usually coupled for flight) and fan cool air through the colony. If shelters were not effective for heat protection, cooling the colony may have been a higher priority for workers. Also, the lull in activity at noon was probably indirectly influenced through competition by the presence of large numbers of honey bees in hot mid-day periods. As well as leaving little nectar in flowers for bumble bees, their physical presence has been reported to cause bumble bees to leave the immediate area (Holmes 1961).

The only major microclimatic fluctuation was that of temperature in session 15 (4 March). On this date, there were higher than usual numbers of bumble bees (both marked and unmarked) recorded in the crop, although not the highest overall. Temperature exceeded 35° C, which is too hot for honey bee activity. Perhaps more bumble bees took advantage of their absence on this day, since their thermoregulatory abilities (Section 2.2.2) enable them to be active at ambient temperatures up to 46° C (Barth 1991).

Marking and reobservation efficacy

As shown in Figure 4.11, the rate of reobservation of marked bees in the field increased from 20% to 100% from the first to second session, then averaged at approximately 75 - 80% over the remaining 17 sessions. Therefore, this marking method was very reliable once powder had penetrated and built up in the nests, and the bees became accustomed to passing through the marking tunnel. No ill effects such as higher larval death rate were observed as a result of the powder, which is specified as non-toxic to humans.

Because the marking method used was very reliable, low reobservation rate of marked bees can not be blamed on the presence of unmarked bees, including non-nest bees in the crop.

Spatial distribution of bumble bees in the crop in relation to their nests; distances and directions

Of marked bees found within the crop, most did forage relatively close to their nests. On average over all nests approximately 81% were observed less than 50 m from their nest. Some

were observed as close as 10 m to their nest. This supports the findings of Saville (1993). Butler's (1951) finding *B. agrorum* individuals no further than 18.3 m from the nest within a crop of red clover matches the 17.5 m modal flight distance recorded in this study. It is not that surprising that at least some do forage close to the nest, but the important question is: where were the rest of them in this thesis study? Butler did not mention reobservation success. The field's shape and size and the positioning of the bumble bee nests in this lucerne work probably influenced the maximum potential flight distances achievable by bees from different nests within the crop, so results should be interpreted with caution since all nest bumble bees were not presented with equal flight distance opportunities. To overcome this in future work, nests should be placed in the centre of larger crops.

The entire crop was observed to avoid the predictable leptokurtic curve of flight distance distributions arising. This mathematical artefact arises when partial sampling methods are used (see definition and description in Section 4.1) A leptokurtic-shaped curve still resulted however (Figure 4.2). One should interpret this with extreme caution in view of the extremely low reobservation rates of marked bumble bees. It does indicate though, that at least some bees do forage close to their nest, and that this is not purely the result of a mathematical sampling artefact.

Individuals could not be followed during their whole foraging bout. Therefore it was not known whether the bees seen in the crop included it as only one part of their bout, or, if these individuals "fixed" their efforts on small areas within the crop. The latter behaviour was recorded by Singh (1948), who found that honey bees restricted their foraging efforts to areas as small as 10 square feet (appoximately 1 square metre) in patches of Birdsfoot-trefoil *Lotus corniculatus*. It is possible that multiple observations of the same individuals occurred between sessions, which were loyal to the same small area within the crop. Individual rather than mass-marking of some bees may have been useful in determining this.

Bumble bee foraging in relation to flower distribution; "hot and cold spots"

The crop appeared relatively uniform in terms of stem and flower density, but Figure 4.5 showed that there was considerable variation. Bumble bees were significantly non-randomly

distributed in the crop. It is not completely clear from this work what the mechanism controlling their aggregated distribution was. Bumble bee densities were greatest on the westward side of the crop and in the top north-east corner (Figure 4.4). Many areas of "bumble bee-hot spots" matched areas of "flower-hot spots", suggesting that bumble bees were "attracted" to dense patches of flowers within the crop. However there was no statistically significant correlation between these parameters. The weak positive correlation however, could mean that a valid relationship exists, but that results were not significant due to very low sample numbers of bumble bees in any crop square.

Edge effects along the west side of the field where bees were concentrated could be because of easier access to the crop along the edge. The lucerne plants were growing around one another and therefore it was physically more difficult for bumble bees to access the centre of the crop compared with the edges. It is unlikely that nectar and pollen rewards differed greatly over the small area. Soil type, light and moisture conditions were the same for the whole crop area.

Differences in reproductive success between nests

Colony size varied markedly in the reared nests as shown in Figure 4.8. There was a relationship between size of nest and number of bumble bees reobserved for each particular colour as one would expect (Figure 4.1). The largest nests; blue, light yellow, dark orange, and light orange accounted for the largest percentages of reobservations. The pink nest, which produced an average number of workers, accounted for the greatest number of reobservations of any colour (23%). This may bias results if bees from different nests "majored" (concentrated their foraging efforts) on different species. Current work at Lincoln University is providing evidence that bumble bees from several nests in the same area do not necessarily forage on the same plant species, or the closest resource. Four *B. hortorum* nests were placed next to a 5 ha field of red clover. So far, results show that in one nest, most bumble bees returned with dark brown pollen loads, suggesting that foragers from that nest are "majoring" on the adjacent 5 ha red clover crop. Bumble bees from the other three nests returned with a wide variety of different-coloured pollens. There are no other red clover crops in the area. Pollen analysis will be used to confirm results (Barron pers. comm.).

Innate behaviour to avoid predation?

This work has provided support to show that bumble bees do not necessarily forage close to their nest, as highlighted by Dramstad (1996a,b). An innately-controlled mechanism to avoid nest-predation as suggested by Dramstad (1996b) may be operating. Spacing out by foragers has been reported as a strategy to reduce predation risk in other species too (Tinbergen *et al.* 1967). It has also been argued though, that foraging is the sole responsibility of bumble bee workers (Waddington & Heinrich 1981). Where bumble bees are native in Europe and the USA, several predators of both foragers and nests exist (see Dramstad 1996b; Plowright & Laverty 1984 for detailed reviews). In addition to predatory mammals (skunks, badgers, foxes etc.), there are internal and external parasites (Alford 1975), spiders and birds (Plowright & Laverty 1984), and "Cuckoo bees" *Psithyrus* spp.. The latter are nest inquilines which live at the expense of true bumble bees by leaving eggs in their nests for the bumble bees to rear (Alford 1975). It would be in bumble bees' interests to "hide" their nests, to prevent advertising activity to would-be predators or parasites. Plowright & Laverty (1984) predicted that pressure to avoid predation should be greater for non-camouflaged animals. In that case, bumble bees which are distinctly coloured, large and noisy, should hide their nests. Their aggressive behaviour if threatened (including a sting defence) and strong flight abilities can protect them out alone in the field as spaced-out individuals. In New Zealand there are no recorded predators. If the predatory theory is correct, that bumble bees consistently do not forage close to their nests, even in the presence of large resources, it will have large implications for management of bumble bee nests for crop pollination. Recent work by Dramstad (1996a) showed that bumble bees from nests placed adjacent to a large strip (ca. 300 m x 2 m) of *Phacelia tanacetifolia* increased their usage of it after the nests had been moved several hundred metres from it.

Resource depletion near the nest?

Lucerne, like many legumes can be frugally rewarding under some conditions (Fairey 1993). Bee requirements for pollen and nectar may not have been met. More profitable rewards may have been offered by alternative sources, such as other crops or weeds in the area. The attractiveness of non-crop plants is discussed in Chapter 5. Bumble bees are very efficient workers, and, in the presence of high numbers of honey bees in the crop, which was relatively

a small one (0.3 ha), as well as eight bumble bee nests, pollen and nectar resources could have been limited. Lucerne in some conditions can be poorly rewarding in nectar and pollen compared with other bumble-bee visited flowering species. Conditions during this experimental period were very dry, possibly resulting in poor nectar flow. Attempts using microcapillary tubes in the field to sample nectar volumes from lucerne flowers failed because either amounts were so minute, or there was no nectar at all. More rewarding flower species beyond the crop could have "attracted" the nest-bumble bees. The attractiveness of non-crop species is a theory pursued in Chapter 5. As the lucerne reached full flowering, more bees were observed in the crop. With such low reobservation rates, it is difficult to say whether this result was a coincidence, or if it was in response to increased pollen and nectar rewards.

Direct competition between bumble bees, and with honey bees ?

Bumble bees are not territorial and will forage closely in one other's presence (Donovan pers. comm.). In this study bees from different nests were observed foraging closely within the same crop grid cells, which supports this theory. This may have implications for the placement of nests in the field at high stocking rates, and where high populations of honey bees exist. In addition to the dry season possibly limiting nectar flow, many honey bees visited the crop and gathered nectar without tripping flowers. It is possible that the small remaining nectar volumes were not enough to "attract" the bumble bees, i.e., a competition effect. The presence of honey bees alone has been claimed to deter bumble bees (Holmes 1961).

Foraging economics- was the lucerne not profitable to bumble bees ?

Optimal foraging theory assumes that individuals strive to maximise gains while minimising costs (Heinrich 1979c).The pollen and nectar energy a bee earns, either for itself or the colony, or both, must not be outweighed by the flight energy cost of gaining that reward. Before the crop seemed profitable to the bees, i.e., when local resources were limited, a 750 m forage flight may have been warranted by a high nectar reward from the weeds found at that distance. This may be because short-tongued bumble bees are generalists, able to forage on several flower species blooming at the same time as the crop and these may be more energetically profitable- through better pollen and nectar rewards, and by being less labour intensive on which to forage (e.g., not requiring tripping). The lucerne was in an exposed area, subject to

frequent strong winds, whereas other species visited grew in more sheltered areas, e.g., purple loosestrife grew in a nearby ditch sheltered by tall trees. Morphologically, some species would be less energetically demanding for a foraging bee. Plants with clusters of flowers allow energy to be saved by crawling rather than energy-expensive flying for example, as would flowers with more robust stems, allowing better support of the bee. Bumble bees use shape and form as long-distance cues. Therefore, tall plants with inflorescences arranged in spikes, such as those of purple loosestrife, could be more attractive to bumble bees than was lucerne. It has also been argued by (Heinrich 1979a) that bumble bees are attracted to spike inflorescences in preference to other species, because their nectar and pollen rewards are more predictably arranged.

Implications of this study for the management of bumble bee nests for pollination of field crops

In addition to providing insight into their potential commercial use in the field, the laboratory-reared nests acted as models for natural nests. This work suggests that the value of nests placed in this lucerne crop was minimal, because so few bumble bees actually visited the crop. The key question though, is how many bumble bees are actually needed to achieve a sufficient level of pollination in the crop? Bumble bees work quickly and transfer large amounts of pollen between plants. This emphasises the importance of combining spatial and temporal information (see model for red clover pollination by Macfarlane & van den Ende 1989) in assessing bumble bee efficacy. Perhaps a few bumble bees visiting the crop may have provided enough pollination. Most of the bumble bees observed in the lucerne were pollen collectors, and the majority of nectar collectors also tripped flowers, effecting pollination as they probed for nectar. Future work could attempt to relate patterns of bumble bee visitation to the distribution of tripped florets. The difficulty would be in finding a field site with no other bumble bees, honey bees, leafcutter bees or alkali bees which could trip flowers. Hot conditions and strong winds can also cause spontaneous tripping.

Future studies

Bumble bee foraging efficiency in more typical, larger paddocks needs to be assessed. It would be useful to repeat this work in crops which flower later in the autumn when fewer competing crops exist. There is evidence that honey bees readily switch to flower species other than the crop the grower wished them to pollinate if more rewarding flower species exist, and must be

managed using special strategies to force them to visit the target crop. Strategies include scent-training and "swamping" (see Jay 1986). Work should be carried out to test whether bumble bees could be manipulated in the same way. Although the communication and learning capabilities of bumble bees are not regarded as being as highly developed than those of honey bees, there is recent evidence to suggest that bumble bees are stimulated to search for specific pollen types by odour and auditory cues (Plowright & Laverty 1984). In view of this, there may be opportunities to manipulate bumble bees to pollinate field crops. Advantages to crop seed production need to be quantified and compared with controls, although because of the multivariate nature of factors influencing the pollination process, this would be difficult to replicate scientifically in the field.

Alternative methods for determining movement patterns of bumble bees include pollen analysis. Examination to determine contents of pollen loads returned to the nest by foragers, or of pollen types in empty nests at the end of the season, should provide a useful method to provide evidence of where bees have foraged (Edwards-Anderka & Pengelly 1970; Prys-Jones & Corbet 1991). For this study, nest entrance collection of pollens from corbiculae of returning foragers would have given a useful measure of the percentage of bumble bees foraging on lucerne, and help to identify other major forage sources. Current work at Lincoln University which utilises pollen analysis techniques to assess foraging behaviour of the specialist long-tongued bumble bee *B. hortorum* , in a 5 ha. red clover crop is a useful progression from the work in this thesis (Barron pers. comm.). Pollen analysis methods will be particularly useful for gauging the specificity of bumble bees to a given crop.

Chapter 5

Pollen and nectar rewards in two plant species visited by *Bombus terrestris*

5.1 Introduction

In the study in Chapter 4 (carried out in 1994) it was found that the reobservation rate of marked bumble bees in a lucerne crop from nests placed adjacent to the crop were very low (< 1%). One factor influencing this non-specificity of bumble bees to the target crop could be related to the presence of other plant species in the area offering better pollen and nectar rewards (see Schaffer &Wratten 1994).

The study carried out in this Chapter (in 1995) involved a survey to compare pollen and nectar rewards offered by the lucerne crop *Medicago sativa* with those offered by purple loosestrife flowers *Lythrum salicaria* , an adventitious plant species in New Zealand, which grew abundantly outside the crop, and on which marked bees frequently foraged. In addition to sighting marked nest bees on purple loosestrife flowers, its distinctively coloured pollen was recognised in pollen loads returned by foragers to the nests on the lucerne crop perimeter. The furthest marked bee observed on purple loosestrife was 750 m from its nest. At their closest point, the lucerne crop and purple loosestrife plants were approximately 10 m apart. Several hundreds of plants of *L. salicaria* plants grew in a ditch extending eastwards from the crop ca. 1.5 km long (Plate 5.1). The presence of this exceptionally dense resource, gave reason to suggest that this is where most of the bumble bees which disappeared from the lucerne crop went to forage.

Considerable variation in plant rewards is possible over time and between sites (Cruden & Hermann 1983).This study was carried out because there was no reliable basis on which to select nectar and pollen production values from the literature for conditions comparable with the study site in Canterbury (see Chapter 4 for site details).

The effect of pollen and nectar rewards on bumble bee movement patterns

Several factors influence movement patterns of pollinators. These may be related directly or indirectly to the insect, the environment, or the plant (see Section 2.5). The work in this chapter focuses on the effect of plant factors on movement patterns by bumble bees. There is evidence to show that pollen and nectar rewards are a key factor affecting movement patterns of bees through foraging decisions made by honey bees (Jay 1986) and by bumble bees (Baker & Baker 1983b; Hartling & Plowright 1979; Heinrich 1979a; Heinrich, 1979e; Morse 1980; Pleasants 1981).

Aspects of pollen and nectar production which influence foraging decisions by bumble bees

The most common parameters reported in movement studies by bumble bees involve responses to nectar volume and nectar concentration (see Section 2.4). Responses to pollen are not well understood. Fewer studies have been performed, probably because it is almost impossible to manipulate as nectar is. Its chemistry is very complex and this differs greatly between flowers species. Although specific pollen collection tasks are carried out by a certain proportion of workers in a bumble bee colony, it is not well understood how bumble bees discriminate, or even if they do, on the basis of pollen rewards when faced with decisions of which flower species to visit (Stanley & Linskens 1974; Free 1993). In this thesis assessing pollen quality components was omitted because very little is known about how bees assess pollen. It is probable however, that the quantity of pollen collected is of great importance. The rate and success of developing brood in the hive depend on a steady supply of incoming protein, particularly in the early stages of worker production, and in the phase of rearing new male and queen reproductives at the end of the season (Woodward 1990). Theoretically, foragers could gain high profits by visiting flowers with lots of pollen, rather than having to spend more energy in flight and flower manipulation visiting several flowers with small amounts of pollen.

Studies exist which suggest insects can discriminate between flowers based on specific components within pollen or nectar. For example, nitrogen content in pollen may be an important factor influencing which flower species a bee chooses to visit (Stanley & Linskens 1974). In addition, the physical surface structure of pollen grains is thought to affect digestibility (Brown *et al.* 1992), and therefore may be another specialist factor determining

forage choice by the bumble bee. Odour cues may also play a role in both nectar and pollen-type recognition (Plowright & Laverty 1984).

In discrimination based on nectar rewards, bees tend to favour flowers whose nectar contain predominantly sucrose rather than other sugar-types (Baker & Baker 1983b) and which are in the 20-50% concentration range (Eickwort & Ginsberg 1980). High sugar concentrations in small volumes of nectar are generally considered to be the optimal choice for maximum profit (Heinrich 1979c). Flowers with high volumes of nectar may not necessarily be the most profitable for bees. As (Prys-Jones & Corbet 1991) pointed out, if the sugar concentration is low, there may be considerable extra effort required to transport large volumes back to the colony, and then evaporate it to a consistency suitable for storage and larval consumption.

Scope of this study

This study deals with basic measurements of pollen quantity, nectar quality and nectar quantity only, because as outlined, understanding of exactly how bumble bees respond to specific components within pollen and nectar (except sugar levels) are not well understood. In addition, the chemical knowledge and laboratory techniques required for such detailed analyses were not available.

Lucerne, purple loosestrife and other bee-visited species' nectar rewards; ballpark figures

Both purple loosestrife and lucerne have been reported to be attractive to bumble bees in terms of pollen and nectar rewards (Levin *et al.* 1971) and (Gurr 1974), respectively. Literature values are highly variable, both from different sites, and because results vary depending on measurement techniques used (see Cruden & Hermann 1983). Some values for sugar content in nectar of some common bee-visited species, including purple loosestrife are given in Table 5.1.

Several authors have measured quality of lucerne nectar . Calculations of average sugar concentrations in nectar range from 15% (Shaw 1953; cited in Free 1993) to 80% sugar (Pederson & Bohart 1953). Mean weight estimates range from 0.34 mg to 1.28 mg nectar per flower per 24 hours (Petkov & Simidchiev 1965 cited in Free 1993). Lucerne is sometimes recognised for its relatively frugal pollen rewards which cost the bee considerable energy to

acquire by tripping the flowers pollen-releasing mechanism, and its viscous nectar which bees find difficult to harvest (Fairey pers. comm.; Langer 1967).

Table 5.1. Sugar content of nectar in some common bee-visited flower species (after Beutler 1949).

Species	**% sugar in nectar**	**mg sugar/flower /24 hours**
Lythrum salicaria L. (Purple loosestrife)	52.	0.05
Lotus corniculatus L. (Birdsfoot-trefoil)	40	0.08
Medicago falcata L. (Yellow lucerne)	33	0.07
Borago officinalis L. (Borage)	53	1.3
Trifolium pratense L. (Red clover)	23	0.08
Trifolium repens L. (White clover)	40	0.04
Phacelia tanacetifolia Benth. (Tansey leaf)	28	0.4

Measurements of pollen quantity in a range of bumble bee-visited species; ballpark figures

Estimates of pollen quantity and quality are limited. Barth (1991) recorded 2.6 million pollen grains in a corn poppy *Papaver rhoeas* (L.) flower, which is considered an extremely good pollen source for bumble bees. Mann (1953; cited in Free 1993) estimated between 8,000 and 13 000 grains in muskmelon flowers *Cucumis melo* (L.). Brown *et al.* (1992) estimated number of pollen grains in red clover flowers *Trifolium pratense* to vary from 33 000 to 129 000, and in broad bean flowers *Vicia faba* (L.; Leguminosae) from 112 000 to 787 000 grains. The presentation of the anthers and the ease with which pollen can be extracted are also important.

Morphology of lucerne and purple loosestrife flowers, and of their pollen grains

Lucerne inflorescences have ten or more small purple flowers each, and there are many inflorescences per plant with corollas 7-12 mm long. Plants flower from November to May (Webb *et al.* 1988). Each flower has five petals of which the inner two form a tight keel which has to be "tripped" (see Section 2.2.2) by the pollinating insect before fertilisation can take place. The keel petals enclose the staminal column of ten stamens and a single style (Free

1993). Pollen grains of lucerne are typical of those of many legumes, and are similar in shape but not size to those of white clover *Trifolium repens*. Lucerne pollen grains are smaller, rounder and smoother compared with those of purple loosestrife pollen grains (Sawyer 1981).

Purple loosestrife is a hairy perennial herb with erect stems growing 1- 2 m tall. Plants are tristylous (a condition in which short-styled, mid-styled and long-styled morphs with reciprocal anther positions coexist so that the sequence of heights in each morph is different). Inflorescences are terminal and branched with a prominent main axis. Flowers grow in whorls of three to seven. Each flower has 6 crimson petals each 9 -15 mm long, and 12 stamens of which six are exserted and six are included (Webb *et al.* 1988). The pollen grains of purple loosestrife are distinctly striped (Erdtman *et al.* 1961; Moar pers. comm.) and are therefore relatively easy to recognise under the microscope. The structure of purple loosestrife pollen grains is distinct from that of the pollen grains of any other plants growing in the local area of the study site (Moar pers. comm.). The distinct striate exines of purple loosestrife pollen grains are illustrated in Plate 5.2.

The aim of the this Chapter was to compare pollen and nectar produced by lucerne with that produced by purple loosestrife flowers in one field site, using the same techniques for both species. It was expected that results would show lucerne flowers to be less rewarding than purple loosestrife ones, and that this could help to explain why most bumble bees in the study in Chapter 4 did not forage in the lucerne crop. In addition to direct pollen and nectar cues, indirect advertising cues from the plant (colour, shape and architecture) are likely to influence forager visitation. Results are discussed in terms of these features.

Specific aims of this work were to measure the following parameters available to bumble bees from each plant species:

- nectar quantity (volume)
- nectar quality (sucrose concentration)
- weight of sugar
- pollen quantity (number of grains)

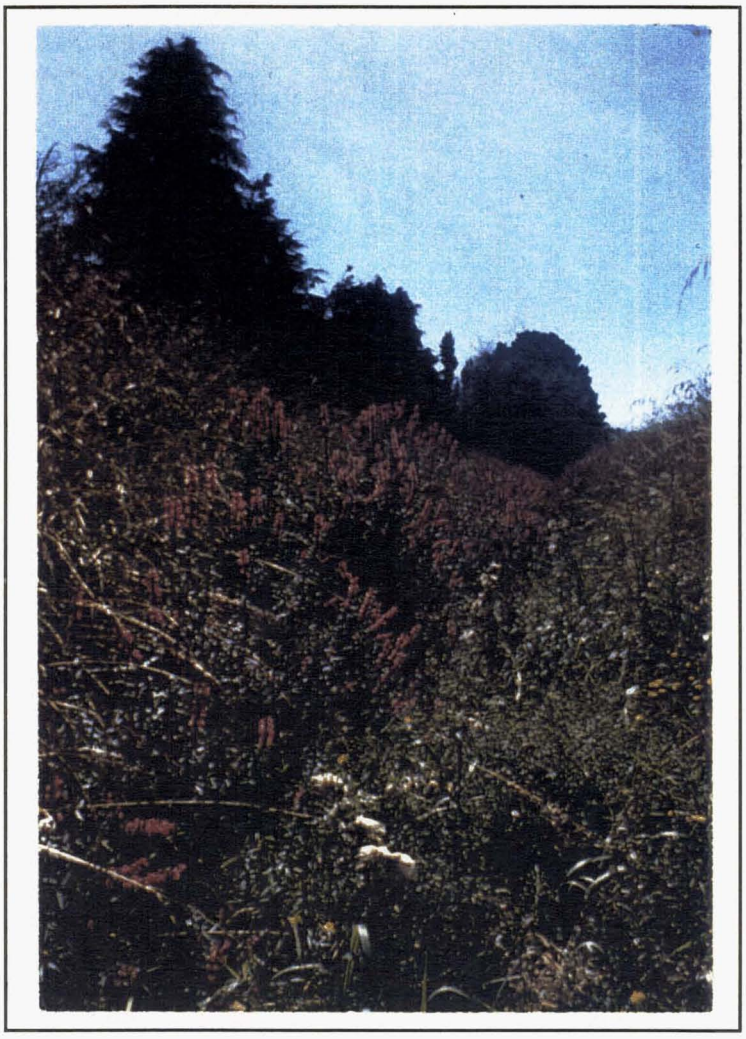


Plate 5.1. Several purple loosestrife *L. salicaria* plants in a ditch near the experimental lucerne crop.

5.2 Methods

Sampling procedure

For all experiments, flower samples from 20 plants of lucerne and 20 plants of purple loosestrife were analysed. Several flowers were sub-sampled from each plant. The temporal patterns of flower opening, anther dehiscence and nectar availability were not known, so for standardisation of both species, plants were covered with bags made of fine-mesh curtain-type fabric (Figure 5.1) for 24 hours to prevent insect visitation. Bags were placed over plants in the mid to late afternoon period when insect activity had ceased, as suggested by Kearns & Inouye (1993). Although standing nectar-crop measurements may have been more indicative of

available rewards to bees compared to the 24 hour accumulation method used, attempts failed to measure the minute quantities present.

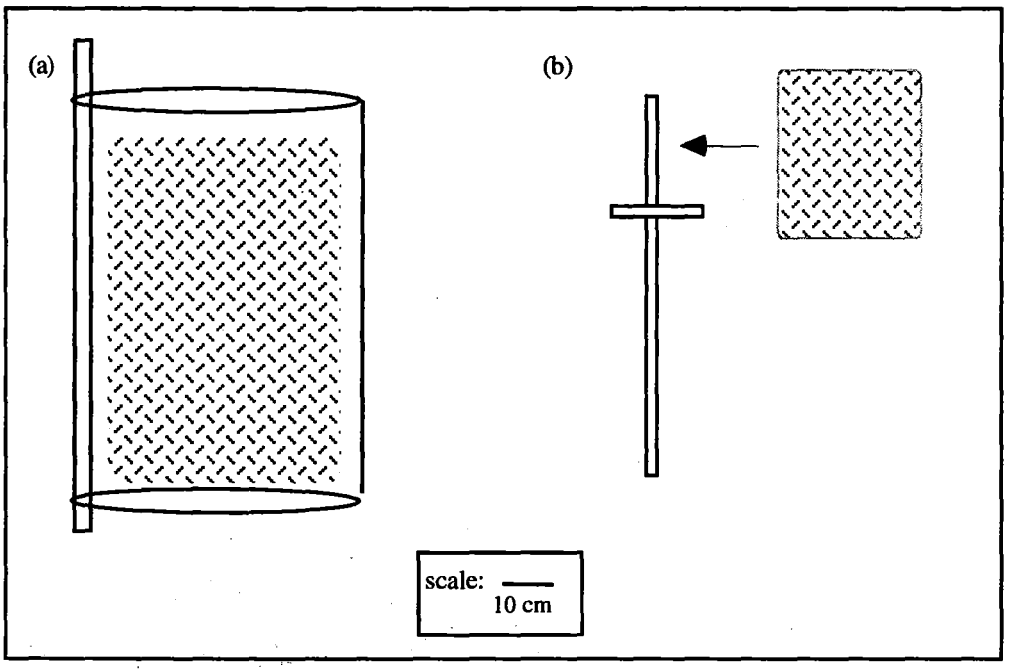


Figure 5.1. Insect-exclusion bag designs for a) lucerne and b) purple loosestrife plants. a) Wire netting cylinder covered with fine-mesh fabric and attached to stake in ground. b) Bamboo cross to attach to purple loosestrife stem with fabric bag to cover the inflorescence spike.

Nectar volume

A technique in which nectar spot diameters on filter paper, are converted to volumes was employed (see Dafni 1992). For detailed methodology see Appendix 5.1. Basic-red fuchsin stain was used to locate the nectaries at corolla bases. For each flower, the corolla was separated from the calyx, and minute amounts (in the order of 0.05 μl to 3 μl) of nectar were extracted using a drawn-out micro capillary tubes. This was performed under a binocular microscope (at 2x magnification) with a cold-light source to prevent nectar evaporation. In flowers for which no nectar was harvested, a blank ("no measurable nectar") was recorded. A new clean capillary tube was used between each extraction to prevent contamination between samples. For each purple loosestrife plant, sampling involved taking one flower from each of five different whorls on the inflorescence spike. Newly-opened flowers only were selected, because these are the ones available for bee visitation. Similarly, for each lucerne plant, one mature flower which had not been tripped, was sampled from each of five inflorescences. The mean volume of these five flower "sub-samples" were used in analyses.

Nectar concentration

According to Free (1993), flowers with protected or tubular corollas tend to have sucrose as the dominant sugar. Purple loosestrife has tubular flowers, and lucerne also has concealed nectaries in comparison with shallow-corolla tubed flower species, whose sugar composition are often dominated by glucose and fructose. Sucrose does in fact constitute a large proportion of lucerne nectar sugars, although glucose and fructose are present (Free 1993). This experiment was therefore based on sucrose standards.

Refractometers are simple and fast to use for measuring the sugar concentrations of many nectars (see Prys-Jones & Corbet 1991). However, they were not found suitable in this work because lucerne volumes in particular were too small to allow a reading. In addition evaporation occurred on the glass refractometer plate, when attempting to pool several flowers' nectar to obtain a reading. A modified refractometer for small amounts was not available. Refractometers are also prone to interference from some compounds, apart from sugars, which have light refractive properties. These interference compounds include amino acids and lipids, and can lead to inaccurate measurements (Cruden & Hermann 1983).

Therefore, a method specifically designed for small amounts of nectar was employed. After the plants had been bagged for 24 hours, they were cut at the stem bases and bought directly back to the laboratory. They were stored in cool conditions with their stems in water, while flower samples were quickly taken. Nectar was obtained using drawn-out microcapillary tubes to harvest nectar from flowers, and "spotted" onto Whatman no.1 filter paper which absorbed the nectar solution. The liquid component evaporated, leaving the solid sugar component in the paper. There is a relationship between light absorbance and sugar content (Dafni 1992). The next stages of analysis involved the redissolving of these dried sugar samples into solutions which could be analysed using light spectrophotometry. Details of the series of chemical reactions which were performed are given in Appendix 5.1. Four flower samples from each plant were sufficient, again selected using the same criteria as for volume measurements.

Light absorbance readings were converted into percentage sucrose concentration of nectar using a calibration curve. This was prepared previously using standard sucrose solutions. Standards were prepared at 10% intervals from 0% (control) to 60%, and two higher-range concentrations were also prepared (80% and 100% sucrose). Based on most literature data, results were expected in the 30% to 60% range, but the 80% and 100% standards were prepared because of some high values also reported. Mean absorbance values from four replicate sets of standards were used to prepare the calibration curve. Several processing stages were involved and it was found in preliminary trials that small differences during any step, could result in large end results. For example, it was found to be important to rotate the samples each 20 minutes in the desiccator stage to ensure equal drying of samples. The assumption was made that any reacting solids were sugar.

Estimating weight of sugar produced

For each plant replicate, mean nectar volume (ml) and concentration (% sucrose) results were combined to estimate the quantity of sugar produced (mg) which was available to bumble bees after the 24 hour period of bagging. An example calculation in which molecular weight and molarity properties of sucrose were used to convert volumes measures to solid weights is given in Appendix 5.2).

Number of pollen grains available to bees

Before counting pollen grains under the microscope, to check that samples were not contaminated with any other pollen types, pollen identification was confirmed with the assistance of a palynologist, Dr. N. Moar, and identification keys (Sawyer 1981; Erdtman *et al.* 1961; Moore *et al.* 1991). Permanent reference slides were prepared using a glycerol jelly and basic-fuchsin red stain technique (see Erdtman 1943; Kearns & Inouye 1993). Plate 5.2 shows a reference slide of purple loosestrife pollen grains. Over all counts, only one non-study species grain was observed under the microscope, indicating that there was no contamination of samples.

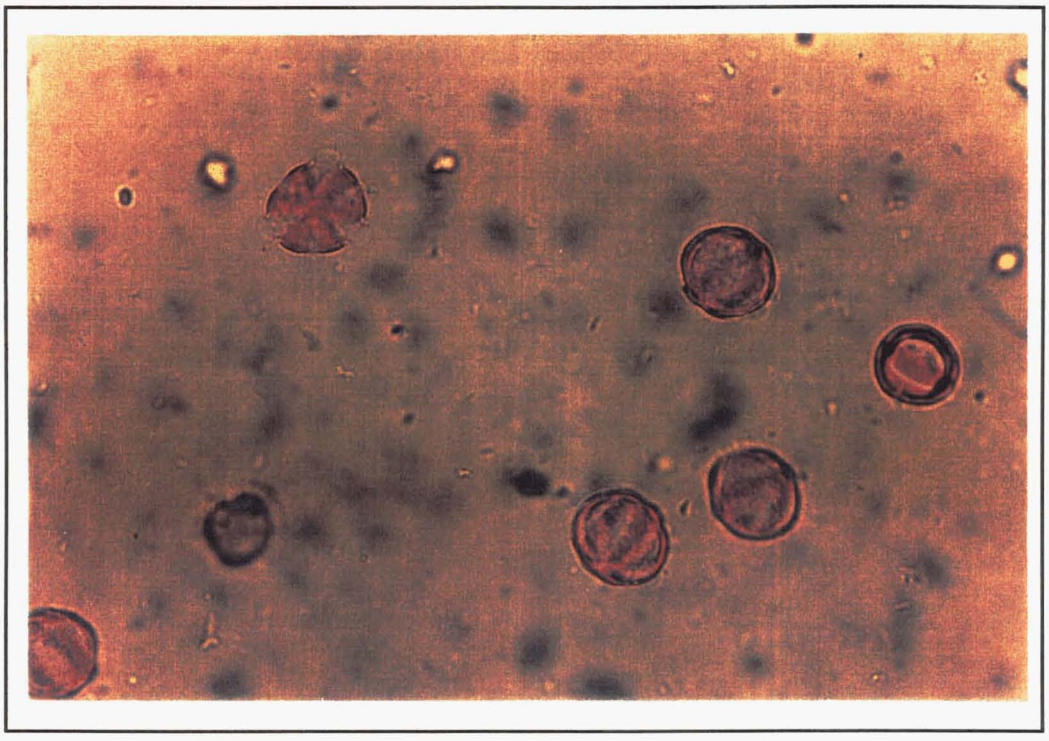


Plate 5.2. Purple loosestrife pollen grains set in glycerol jelly and stained with basic fuchsin red on a reference microscope slide (x 400). The longitudinal striate patterns("colpoid streaks") are characteristic of this species' pollen grains.

Pollen grains were counted using a blood-cell count plate ("haemacytometer"), which is a common method used by pollination ecologists (see Dafni 1992; Kearns & Inouye 1993). For details of methodology and example calculations to convert microscope counts to total pollen grains produced per flower see Appendix 5.3.

For each pollen count sample, mature but undehisced anthers were removed from one flower from each of the 20 bagged plants for each species. Although purple loosestrife flowers each have 12 anthers, field observations showed that only six of these offer pollen at one time. This was taken into account in calculations of pollen available to bees at any time. For lucerne, all 10 anthers were sampled, because when a bee trips the lucerne flower mechanism, all anthers split simultaneously. Some lucerne flowers' on dissection burst their anthers, shedding pollen onto inner petals. Therefore, when this occurred. the inner petals were included in samples so as not to lose any pollen grains. These did not appear to affect the results in any way compared to samples in which petals were not included.

Anthers were removed using a fine-pointed metal probe, under a binocular microscope at a magnification of 8 x, then transferred to a small test tube where they were smeared on the inside wall of it to release the pollen grains. Test tubes contained a solution of ethanol, methylene blue dye, and detergent which prevented grains clumping together (for method details see Appendix 5.3) .

From each test tube solution, grains were counted in four sub-samples. Thorough mixing was carried out using a vortex mixer between each sample to keep grains as evenly distributed as possible in the solution. The metal probe and forceps used to handle anthers, were thoroughly checked for remaining pollen which could contaminate subsequent samples. Pollen counts from each sample were converted to bee-available pollen per flower based on the number of anthers sampled, the number of anthers presented in flowers, and the volume specifications of the blood cell count plate used. For this work a model Mod-fuchs rosenthal B. S. 748 Hawksley Cristalite; 1/16th mm^2, 0.2 mm was used. An example calculation is given in Appendix 5.3.

Statistical analysis

Statistical analyses for all investigations involved the comparison of median values using the non-parametric Rank sum (Mann-Whitney-*U*) test. The 20 plants of each species were regarded as replicates. Several flowers were sub-sampled from each plant to give mean pollen and nectar values per plant, and these means were analysed. Results are expressed per flower. Error terms presented are 95% confidence intervals limits (95% C.L.).

5.3 Results

Nectar volume

A significantly higher proportion (0.71) of all purple loosestrife flowers sampled contained nectar that could be measured (> 0.05μl), compared to lucerne flowers (0.60) ($\chi^2 = 16.28$; $P < 0.001$). The median volume of nectar produced by lucerne over 24 hours was 0.13 ± 0.023 μl and by purple loosestrife 0.17 ± 0.054 μl (not significantly different; $P > 0.05$) (Figure 5.2).

Individual sample volumes per flower ranged from 0 μl to 0.650 μl for lucerne and from 0 μl to 2.95 μl for purple loosestrife.

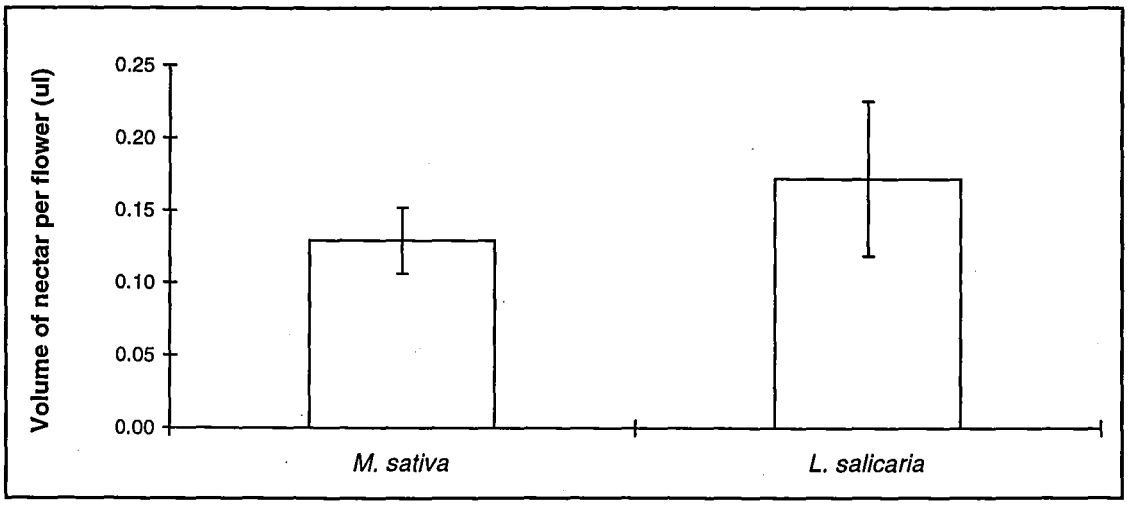


Figure 5.2. Median nectar volumes in lucerne and purple loosestrife flowers after being caged for 24 hours (± 95% C.L.) ($P > 0.05$).

Nectar concentration

The median nectar concentration for purple loosestrife (54.8%) was significantly higher ($P < 0.01$) than that for lucerne samples (28.5%) (Figure 5.5). Results ranged from 0.01% to 94.9% sugar content for purple loosestrife nectar and from 0.00% to 89.9% for lucerne nectar. Figure 5.4 shows the frequency distribution of nectar values in the two species.

It was assumed that the relationship between light absorbance and sucrose concentration should be linear, and extend through the origin. The line of best fit established was: $y= 0.012+0.007x$ ($r^2= 0.79$) (Figure 5.3). Readings from one lucerne sample and from three purple loosestrife samples exceeded 100% sucrose concentration which is impossible. These samples were discarded. It is likely that solid dust particles were attracted by static electricity to the plastic cuvettes, and that these interfered with the spectrophotometer's light beam.

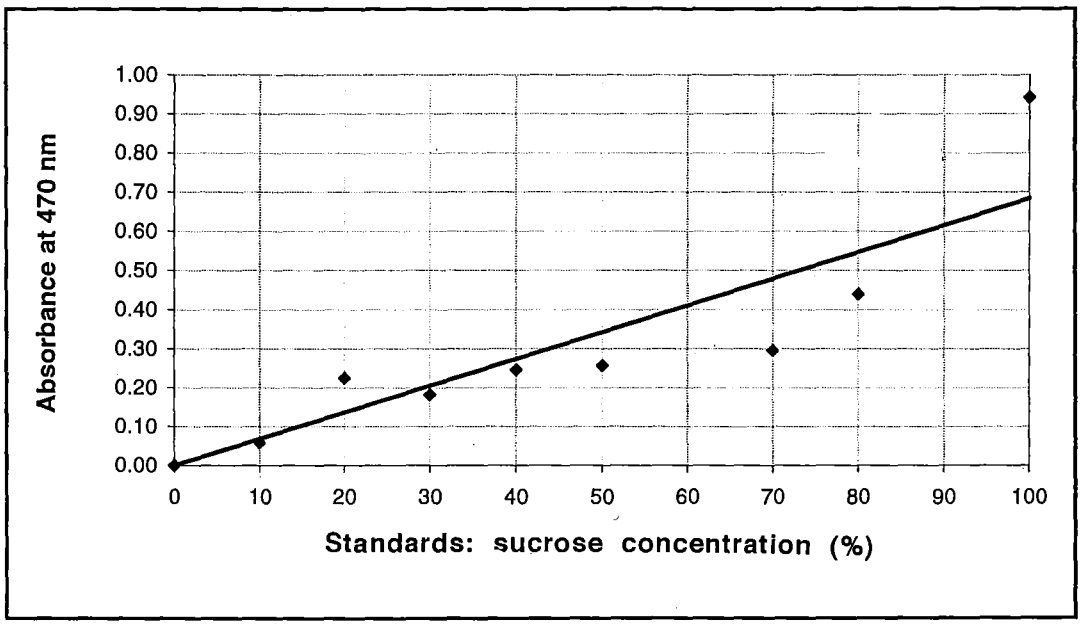


Figure 5.3. Calibration curve to convert absorbance readings from nectar samples in solution to sucrose concentrations.

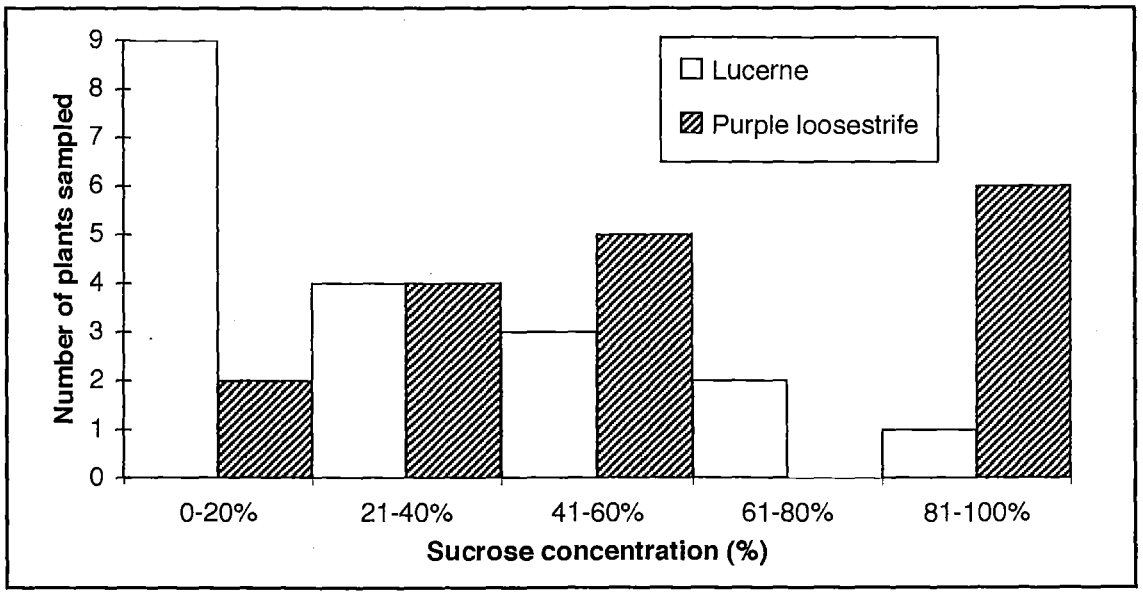


Figure 5.4. Frequency distribution of sucrose concentrations in nectar of purple loosestrife (n=17) and lucerne (n=19) flowers.

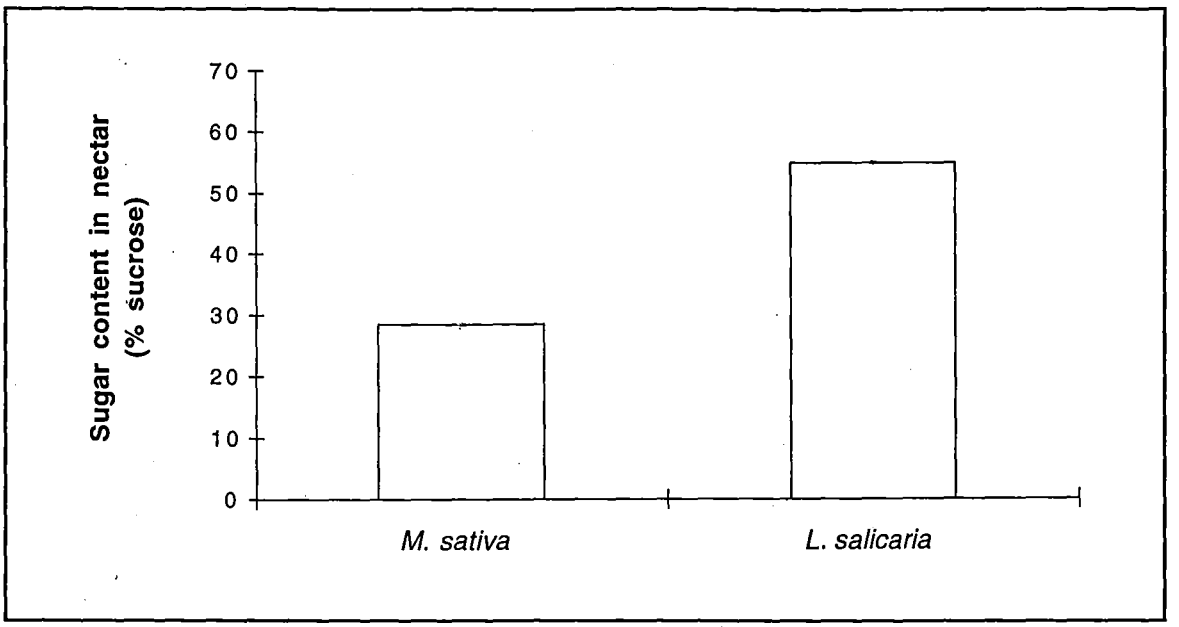


Figure 5.5. Median sugar concentration values in nectar of purple loosestrife and lucerne (n= 17 and 19 respectively)($P < 0.01$).

Estimated weight of sugar produced

There was no significant difference in the weight of sugar (mg) produced between lucerne (0.059 mg ± 0.0163 mg) and purple loosestrife (0.074 mg ± 0.0150 mg) per flower ($P > 0.05$). Estimated sugar weights ranged from 0.0 mg to 0.20 mg for purple loosestrife, and from 0.0004 mg to 0.23 mg for lucerne. See Appendix 5.2 for example calculation.

Number of bee-available pollen grains produced

There were significantly more pollen grains produced by purple loosestrife flowers (17 670 ± 1044) than lucerne flowers (9625 ± 673) after 24 hours ($P < 0.001$)(Figure 5.6). A frequency distribution of total pollen grains produced (Figure 5.7) illustrates overlapping ranges of each species, but with only lucerne values in the low end of the range (0 to15 000 grains/flower) and only purple loosestrife in the highest end of the range (10 000 to 30 000 grains/flower). Total number of pollen grains produced per purple loosestrife flower ranged from 10 670 to 27 750, and per lucerne flower from 4722 to 16 390 pollen grains.

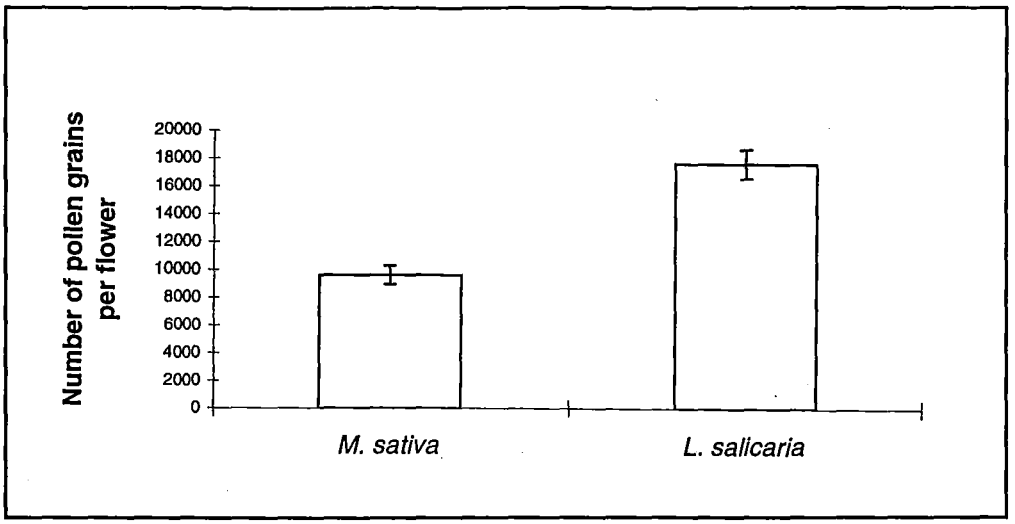


Figure 5.6. Estimated total amount of bee-available pollen produced by lucerne and purple loosestrife flowers over 24 hours ($P < 0.001$).

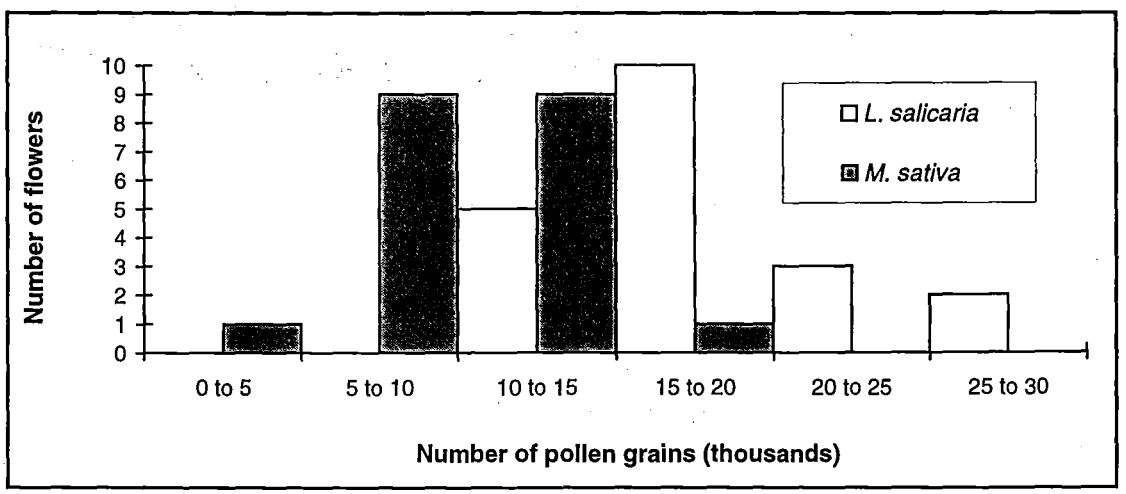


Figure 5.7. Frequency distribution of the range of total pollen grains produced in lucerne and purple loosestrife flowers over 24 hours.

5.4 Discussion

This study showed that pollen and nectar rewards differed significantly between the lucerne crop and the non-crop species, purple loosestrife. On their own, the data presented do not prove that food rewards were the governing factor causing the non-specificity of bumble bees to the lucerne crop observed in Chapter 4. However, because several authors have reported that food rewards greatly influence honey bee (Jay 1986) and bumble bee foraging decisions (Pleasants 1981; Heinrich 1979e), food rewards are likely to have played a significant role in

the patterns observed in Chapter 4. The following discussion considers the potential relationships between the movement patterns observed in Chapter 4 and the level of pollen and nectar rewards offered by the plants measured in this study.

Nutritional requirements of foraging bumble bee workers and their colonies

Foraging workers need enough energy to maintain their flight to and from their nest to the forage site, and within the forage site. In addition they must attempt to make an energy profit and carry back as much pollen and nectar as is profitable to feed developing brood in their colony. How foraging bumble bees firstly discriminate between which flower to visit is not well understood, but it is believed to be a combination of scent, colour and plant architecture (Plowright & Laverty 1984). After visiting a flower species for the first time, the rewards gained by the bee greatly influence their likelihood of visiting the same species again (Heinrich 1979e).

Concentration of nectar sugar is an important factor influencing bee flight distances (e.g., Heinrich 1979a). Results from this study showed statistically significant differences in sugar concentration of nectar produced by purple loosestrife and lucerne flowers. Although these median values were significantly different, values ranged widely between individual flower samples. For both species concentrations less than 20% sucrose and greater than 80% sucrose were recorded (Figure 5.4). Considerable within species variation observed for concentration and volume is usual according to Cruden & Hermann (1983).

Foraging bumble bees quickly respond to changes in colony requirements , and adjust their pollen and nectar collecting habits accordingly (Plowright & Laverty 1984). Bumble bees appear to be sensitive to a certain "target-value" of a colony-level energy requirement (Cartar & Dill 1990). When bumble bee nests were placed in the field, after experiencing *ad libitum* feeding in the laboratory, workers in this study may have been stimulated to collect as much sugar as possible to supply energy quickly to the colony. The study in this thesis Chapter showed that sugar concentration in nectar of purple loosestrife was significantly higher than in that of lucerne. Therefore, the purple loosestrife could have provided these high energy requirements to the bumble bees from the laboratory-reared nests placed in the field.

Pollen requirements

Brown *et al.* (1992) assessed *Vicia faba* and *Trifolium pratense* as forage crops for *Bombus hortorum*, and through calculations based on what reports from other authors on what bumble bees require, estimated that both crops fulfilled bumble bee nectar and pollen requirements. The estimated number of pollen grains in red clover flowers varied from 33 000 to 129 000, and for broad beans from 112 000 to 787 000 grains per flower. In this study, lucerne pollen grain numbers ranged from 722 to 16 390 per flower and for purple loosestrife from 10 670 to 277 504 per flower, suggesting calculations made were correct since numbers are in the same ballpark as the work of Brown *et al.* Without further research however, it is not possible to say whether the lucerne pollen met bumble bee requirements.

Brown *et al.* (1992) highlighted that in addition to pollen protein content, amino composition, and actual number of pollen grains produced, ease of digestibility is of value to the bee. Digestibility is affected by the pollen grain's surface Stace (1987) cited in (Brown *et al.* 1992). Brown *et al.* (1992) concluded that the smooth pollen grain coats of red clover and beans may have contributed to their attractiveness to *B. hortorum* in their work. In this thesis study, lucerne, being closely related to red clover, also has smooth grains. Of the low number of bumble bees that visited the crop in Chapter 4, most of them were foraging for pollen. Some workers may have majored on lucerne specifically for its pollen, rather than nectar, discriminating on digestibility factors.

Although most of the bumble bees in the study in Chapter 4 were collecting pollen in the lucerne crop, they were calculated to constitute only a very small number (< 1% on average) of the total bumble bees which were out foraging from their nests during reobservation periods. The division of labour which exists in bumble bee colonies is flexible in response to changing colony needs depending on the stage of development (Plowright & Laverty 1984). For example, in egg laying and brood rearing, large amounts of pollen protein are required. Purple loosestrife, which this study showed, produces approximately double the amount of pollen per flower that lucerne does. Therefore most of the bumble bee workers in this study could have foraged on purple loosestrife because it was capable of supplying larger amounts of pollen than the lucerne. It is expected that the nests placed in the field, during the experiment would have

been at their maximum requirement level for pollen protein during the flowering period of lucerne. Nest traffic data and nest dissection data (in which cells of all castes were counted) from Chapter 4 showed that during the period February to March there was strong colony growth and reproduction.

Economics of getting to, and foraging on different flower species

While bees do not remove all the pollen and nectar present, they could theoretically gather more pollen grains for a similar effort expended if one flower species offers significantly more than another, and especially if it is more easily available. Handling efficiency, and thus profit will depend on bees ability to intake more energy than that expended in getting it (Harder 1986). Purple loosestrife offered significantly more pollen than lucerne. Figure 5.6 showed that on average, approximately double the pollen was produced by purple loosestrife than lucerne flowers. In addition, purple loosestrife flowers do not require tripping as lucerne flowers do. As Langer (1967) pointed out, many insects can neglect lucerne flowers based on its "low level of pollen attractiveness" combined with the explosive release of the staminal column. It is possible, that lucerne is not actually as attractive to bumble bees as was previously concluded (see Gurr 1974), particularly in the presence of alternative species offering more profitable nectar and pollen rewards. Jay (1984) emphasised the non-specificity of honey bees to crops if "richer" pollen and nectar rewards exist in the area, and how they would readily switch between forage plants based on pollen and nectar "profits". *B. terrestris* may exhibit similar behaviour, because, like honey bees, they are short-tongued generalists, able to gather nectar from a wide range of flowers with shallow corolla-tubes, commonly found in farmland areas.

High concentrations of sugar presented in small volumes of nectar by flowers would theoretically, be highly rewarding for bumble bees. Surplus water content, which is relatively heavy would not have to be transported by the bumble bee. More sugar would be acquired per visit, and less visits would be required to flowers with high sugar concentrations in nectar to achieve a given amount for the colony compared to flowers offering more dilute nectar. It might therefore be more efficient for the forager to visit flowers with concentrated nectar but less volume than flowers with high volumes of dilute nectar. The bumble bees in Chapter 4 may have foraged on the purple loosestrife in preference to the lucerne because purple loosestrife

nectar had significantly more sugar per volume unit compared to lucerne (Figure 5.5). However no conclusions can be made concerning possible bumble bee responses to volumes of nectar, because this work showed no significant difference in volumes of nectar, or extrapolations from it to weight of sugar produced between flower species. In more concentrated nectars, energy could also be saved from drying down the nectar later for use in the nest, e.g., for larval food and honey storage, although Prys-Jones & Corbet (1991) cautioned not to assume that bumble bee larvae thrive in highly concentrated nectar, and that considerations of water balance should be considered. Perhaps bumble bees may prefer to collect nectar from flowers which offer large volumes, in certain cases if the colony requires more moisture. Future work could assess water balance relationships with loads being returned by foragers, since Plowright & Laverty (1984) mentioned that foragers respond quickly to changing food demands of the colony.

Beutler (1951) concluded from studies of honey bee movement that the critical cost is not the energy used in commuting but the time that could have been used to collect more food energy. Beutler found that most honey bees were only willing to travel up to 1 or 2 km from their nest to a rich sugar solution (70% concentration) when it was placed at increasing distances from the hive. Although a few bees visited the solution at 3 km away, Beutler concluded that so few bees did so because the time taken to fly there would have limited their collecting power so much, that they should only to go far distances if "desperate". Long flight distances by bees therefore, must be warranted by high energy profits in terms of pollen and nectar energy gains, to outweigh the opportunity-cost of not visiting flowers close to the nest, which although may be less rewarding, also "cost" less in flight time to obtain. Heinrich (1979a) also concluded that foraging bees should try to keep flight time and distance to a minimum in order to maximise profits. Perhaps in the study in this thesis Chapter, the higher sugar concentrations of purple loosestrife than lucerne flowers, were responsible for the non-specificity of bumble bees to the lucerne crop and influenced them to forage up to 750 m from their nest on purple loosestrife plants.

Results from experiments such as Beutler's (1951) would be useful to gain information on bumble bee patterns of movement in relation to nectar and pollen rewards. For example,

whether they have a threshold level for flight distance based on rewards obtainable, as Beutler showed for honey bees. However, because bumble bees do not appear to recruit new foragers on discovery of a rich sugar source (see Brian 1954a; Michener 1974) these experiments have failed.

"Lucky-hit" nectar rewards

A reasonable reward encourages repeat visits whereas a poor reward or none at all might discourage further visits by a pollinator (Cruden & Hermann 1983). "Lucky hit" nectar rewards were described by Southwick (1982) and may greatly influence future foraging decisions in species that do not consistently offer concentrated nectar. The purple loosestrife in this study was shown to offer a significantly larger proportion of flowers with any nectar at all, and also a higher proportion of flowers with very high sugar concentrations compared to lucerne (Figure 5.4). An initial lucky hit on a very "rich" flower may have influenced bumble bees to return several times to purple loosestrife in the hope of a repeated high sugar reward. Cameron (1981) presented evidence that bumble bees can leave a chemical marking system on highly-rewarding flowers. This may also have lead to several foragers going to purple loosestrife, and other more highly rewarding plants outside the crop in this work.

Indirect effect of microclimate on bumble bee foraging patterns: pollen and nectar changes

Temperature and moisture affect nectar secretion. Most research agrees that nectar concentration increases and volume decreases with evaporation (Cruden & Hermann 1983). In the current work, the unusually dry conditions which prevailed may have affected colony demands by there being little excess moisture for nectar production (Clifford pers. comm.). The crop was not irrigated and rainfall was lower than average for the area. For purple loosestrife, growing in the ditch with more available moisture is likely to have resulted in the larger volumes recorded in some purple loosestrife samples. Although there was no significant difference recorded for nectar volumes (median values) produced between lucerne and purple loosestrife plants in this study, results showed that a greater proportion of purple loosestrife flowers sampled had nectar that could be extracted by the techniques employed compared with lucerne samples. Also, volumes in purple loosestrife flowers samples ranged to three times the volumes found in the highest lucerne flower sample. Bumble bees may have preferred to visit purple loosestrife

because the probability of getting any nectar at all was higher, compared with going to lucerne. In addition, the sugar concentration in purple loosestrife flowers was significantly higher than in lucerne flowers.

It is possible that covering the plants with insect screening material in this study altered the light, humidity and temperature conditions within the enclosures, and hence affected sugar concentration and volume of nectar produced by plants. However, Cruden & Hermann (1983) showed that mosquito netting (similar to the fabric used in this work) had the least effect on microclimate factors of many bagging materials. In this thesis study, although the sugar and volume production results gained may not match exactly those, had the plants not been covered, they still provide relative measures of nectar quality between species.

Bumble bee foraging strategies; "Majoring and minoring"

Bumble bees are known to visit several different flower species during one foraging bout. "Majoring and minoring" (Section 2.2.2) is a behaviour which has been observed for many bumble bee species in many different environments (see Heinrich 1979b). Bumble bees tend to concentrate their efforts on one (major) species, while periodically sampling (minoring on) a wide range of other species (Woodward 1990). This strategy allows the constant monitoring of available food sources. Because pollen and nectar rewards change according to environmental cues between and within seasons, and even within single days, one could expect the flower species which bumble bees "major" on, to regularly change in response to reward changes. For example, in the work in this thesis, pollen might have been collected in the morning from lucerne and in the afternoon from purple loosestrife or another combination of species for example. Most reobservation sessions were carried out during peak bumble bee foraging activity periods each day (see Section 4.2). The diurnal cycle of pollen and nectar reward presentations by lucerne and purple loosestrife plants was not known in this work, which is why both species were covered for 24 hours. However, Loper *et al.* (1971) showed that the emanation of flower volatiles in lucerne followed a daily cycle with aroma release between 1100 and 1400 hours. One would expect that aroma release is a cue for pollinator visitation. If lucerne is highly attractive to bumble bees, they might be expected to major on it during their diurnal peak activity period. Most reobservation sessions in Chapter 4 were carried out

between 1500 and 1800 hours (see Section 4.2), which is close to the times cited by Loper *et al.* (1971) for release of aromatic cues from lucerne (1100 and 1400 hours). This provides a basis to expect that if bumble bees were majoring on lucerne at any time, they should have been present in afternoon reobservation sessions. Because very low numbers (< 1%) of bumble bees from the nests were observed in the lucerne crop at these times, suggests that lucerne was not a preferred species of bumble bees.

Spatial and temporal arrangement of flower rewards; potential effects of plant architecture

Factors influencing the volume of nectar produced, and its availability to bees include the number of flowers per floral unit, the distance between the floral units, and flower density (Cruden & Hermann 1983). Both lucerne and purple loosestrife flowers grew in architecturally complex arrangements. It is likely that neighbouring plants within each species affected each other's nectar production through light and moisture microclimate effects. This could account for the wide range of nectar concentrations and volumes within each species. Bumble bees could save flight costs in both species because the flowers were close together. The tangled growth habit of lucerne could impede access of bumble bees to flowers, whereas the open, spike arrangement of purple loosestrife inflorescences may have provided easier access.

Individual plant architecture and location of plants in this work may have indirectly influenced bumble bee patterns of visitation. Bumble bees can discriminate between different plants to visit based on their height (Schaffer & Schaffer 1977). It has been suggested that some plants have developed specific statures to promote visitation by certain pollinators (Plowright & Laverty 1984). Levin (1973; cited in Plowright & Laverty 1984) specifically mentioned that pollinators have been shown to discriminate between forage plants, using height characteristics of purple loosestrife.

The arrangement of flowers within each plant may also have also influenced bumble bee movement patterns. A spike composed of several flowers each with poor quality nectar may be as profitable as single stalked flowers spaced apart each with high quality nectar, simply because the bees energy travel costs are lower on the spike plant. Crawling between flowers to collect pollen and nectar, rather than flying can save considerable energy for foraging bees

(Heinrich 1979a). Purple loosestrife provides robust spike inflorescences which can support crawling bumble bees. In addition, the plants were growing in a sheltered ditch. Less wind may have been directly favourable for foraging activity, and indirectly favourable to bumble bees by providing a warmer microclimate resulting in higher sugar content in flower nectar.

Competition between bumble bees and honey bees for available nectar in the crop

Honey bee densities were measured in the crop in 1994 (see Chapter 4) and far out-numbered bumble bees (thousands compared with tens, respectively). High numbers of honey bees visited the lucerne crop and took nectar without tripping flowers. This might have had an indirect effect on bumble bees movement patterns through decreased or no nectar left in the flowers. Alternatively honey bees may have or a direct influence on bumble bees through their physical presence (Donovan 1994 pers. comm.). Bohart *et al.* (1967; cited in Free 1993) performed a series of experiments to look at how populations of wild bees and honey bees interacted with each other and found that when honey bees were increased on lucerne plots, wild bee numbers (including bumble bees) declined to half their original number.

Implications for management of bumble bees for pollination of field crops

The implications for management of bumble bee nests for crop pollination are considerable if bumble bees are not specific to the target crop (as demonstrated in Chapter 4). Honey bees used for the pollination of lucerne crops have been found to rapidly respond to other non-crop pollen and nectar sources (see Vansell & Todd 1947; Jay 1986), and Free (1993) stated that if other crops are abundant, honey bees may fail to collect any lucerne pollen at all. Both agronomic and pollinator-management strategies exist to improve visitation rates by honey bees to lucerne crops. These include swamping the area with so many colonies that at least some bees are forced to visit the crop as alternative forage sources are saturated, feeding colonies with supplementary sugar solution to encourage them to collect pollen, and scent-training colonies to stimulate bees to forage for a specific pollen type (see Jay 1986; Free 1993). Perhaps it may be possible to manipulate bumble bees in similar ways.

"It is generally accepted that bumble bees do not communicate, at least in the same way that honey bees do which are more highly socially developed" (Michener 1974). However, recent

evidence exists that bumble bee workers may respond to odour cues on foragers returning to the nest, or to odour cues left in the forage patch in the field which could influence them to collect a specific pollen or nectar type (Plowright & Laverty 1984). In light of these reports, there may be more opportunities than previously thought to manipulate bumble bee colonies for field crop production using scent training techniques currently practised to encourage honey bees to visit crops they otherwise would not (see Jay 1986).

In summary, this study showed that lucerne was less rewarding compared to purple loosestrife in terms of amount of pollen produced and concentration of sugar in nectar, and has discussed how these differences could explain why most bumble bees did not forage on the lucerne crop, but many bumble bees foraged on the purple loosestrife in the study in Chapter 4. However, there was no empirical correlation between movement patterns and foraging preferences provided in this work to prove this. Future work should address the relationships between bumble bee pollen and nectar requirements, and the degree to which they will adjust their foraging strategies to achieve these requirements. In addition, applied research aimed at understanding whether it is possible to manipulate bumble bees to be specific to a target crop, which they would not otherwise visit, is most appropriate in view of new evidence reported that bumble bees may be more responsive than previously thought to odour cues conveyed by fellow workers. More research is needed on how bumble bees discriminate between forage plants based on the amount and components of nectar, and particularly of pollen.

Chapter 6

General discussion and conclusions

This thesis has presented two farmland examples in which bumble bee-pollination could potentially be improved through management to meet both conservation and commercial goals. Where and how far bumble bees fly to forage determines the degree of pollen flow and therefore gene transfer between different flowers, plants or isolated plant populations. The work undertaken in this thesis has reinforced the need to address aspects of space usage by foraging bumble bees for which knowledge is currently limited in several areas (outlined in Chapter 2). Most previous bumble bee behaviour studies have assessed temporal aspects of foraging only. The general aim of this thesis was to improve understanding of how bumble bee use the space available to them. Particular emphasis was placed on landscape-scale studies and movement of bumble bees from their nest. These two aspects are the most poorly understood, but have the most implications for farmland plant species success whether for conservation or commercial purposes.

This work has highlighted how extremely complex the pollination process is, and how difficult it is to correlate patterns of bumble bee movement with it. Waddington (1983b) alluded to the many intrinsic and extrinsic factors which are superimposed onto an environmental background that influence and complicate the pollination process. Multivariate statistical analysis to correlate simultaneously operating factors known to influence pollinator behaviour, with the movement patterns observed, was beyond the scope of this thesis. However, single factors considered include climate, resource abundance and distribution, competition with honey bees, diurnal patterns of activity, resource architecture and location, and nest location.

Preservation of traditional Norwegian mountain meadows (Chapter 3) and production of high-yielding legume seed crops on the Canterbury Plains of New Zealand (Chapter 4) involve very different goals. Yet the need to understand pollinator movement in order for management intervention if necessary, is applicable to both. The third experimental chapter (Chapter 3) was an extension of Chapter 4. Pollen and nectar rewards have been shown to influence bumble bee movement patterns. Rewards from a non-crop species on which bumble bees appeared to

forage on in preference to a target crop were compared, in an attempt to explain, at least partly, their non-specificity to the target lucerne crop.

The two farmland habitats enabled the study of movement at several scales ranging from movement between single flowers to movement over several hectares at the landscape level. The importance of assigning different studies to a theoretical framework of different scales has been realised in this thesis. Bumble bee movement and resource distribution patterns have been considered in relation to the pollination process between flowers, between patches, and over large areas in the order of several hectares. There are different implications for results at each scale. Therefore, although there are several factors in common between studies, it is more appropriate to discuss and make conclusions for each study separately.

Small scale movement between flowers and within a patch

Movement between flowers and over the whole patch was random in regard to direction for all individuals analysed and for all pooled results. Because of the large number of possible factors controlling directionality between flowers, more work using artificial flower arrays where specific parameters can be controlled would be useful for future work. Whether bumble bees can learn and memorise their orientation in response to resource distribution and abundance is of particular interest. Although directionality may have limited significance on a small scale within patches for the success of plant populations in general, understanding what bumble bees respond to and how, is important biological information. For example, if they behave in a completely innately-controlled way, regardless of resource, there would be large consequences for the management of bumble bees through human intervention.

Conclusions of small scale work

- Resolution of measurement either between flowers, or over the patch through 1 m^2 divisions produced the same results, indicating that exact resolution for measurement of directionality within patches may not be as critical as expected.
- The consistently random patterns in regard to direction exhibited by bumble bees foraging within a patch are likely to be caused by "area-restricted searching behaviour" caused after encountering a rich resource on a section of the bees' foraging route or "trapline".

Medium and large scale movement in Norwegian meadows

In the study between patches of *Aconitum septentrionale*, results were limited by low reobservation rates of marked bumble bees. This was thought to be due to the generalist foraging nature of the bumble bee species marked, and as a result of an individual coding system being unreliable. Despite this, there was a trend for bumble bees visiting these patches to remain loyal to one, or two patches close together. This could be part of a recognised bumble bee foraging strategy known as "traplining" in which certain patches are repeatedly visited on one section of their foraging bout sequence. Future work needs to assess whether this strategy is detrimental to plant success through reduced pollen flow between distant populations.

Studies within and between meadows in Norway revealed surprisingly restricted movement by bumble bees. This work probably involves an element of sampling artefact because of the transect method employed. The leptokurtic distribution could partly represent decreasing probability of reobservation with increasing distance from the marking points of bees. However, there was a marked sharp drop in reobservations after 50 m from marking points and reobservation rates were high, suggesting that bumble bees do restrict their search areas once reaching a forage area. Very few bumble bees crossed between meadows which may have implications for low levels of pollen cross-over between plant populations in different meadows. Further research is needed to determine the threshold level of pollination plant populations require to maintain genetic stability.

Future work is needed to assess whether pollination is in fact a limiting factor to the success of plant populations in the Norwegian meadows studied, and whether any shortfalls are linked to habitat fragmentation effects. Many patterns observed in the Norwegian study can be explained in terms of recognised bumble bee foraging strategies (largely innate behaviours) although further detailed testing would be necessary to confirm this. Further research on what level resource abundance and distribution can influence pollinator movement patterns is essential.

The effect of physical landscape features on movement patterns of bumble bees is a particularly worthy aspect for further investigation. Previous work has suggested that honey bees tend to disperse more widely within physically homogeneous compared to physically heterogeneous

landscapes (see Gary *et al.* 1977). Plowright & Galen (1985) recognised that bumble bee movement is affected by landmarks at a small scale, and that there is a need to extend this to large scale studies. Work by Fry & Robson (1994) on butterflies and by Evans (1996) on hover flies showed that barriers such as hedgerows and fences as low as 1 m in height can significantly restrict movement. Bumble bees in the Norwegian meadows were surprisingly restricted in their movements, compared with nest bumble bees in a commercial crop environment which dispersed widely (Chapter 4). The forest-surrounded meadows were more architecturally complex, as was the land topography itself compared with the flat and relatively featureless Canterbury Plains environment. Perhaps orientation using long distance cues is more important than previously realised for bumble bees. Individuals were sometimes seen going into the "wrong" colour nest in the New Zealand study, suggesting disorientation. However, these studies are not directly comparable because one involves movement from the nest, and the other movement once bees reach their forage destination. These aspects should not be confused.

Improved methodologies for tracking bee movement using radar which are currently being developed, and trialled for field use at Rothamsted Experimental Station in the UK would revolutionise large scale movement studies if successful (see Hornsby 1996; Riley *et al.* 1996). Ideally equipment must be developed which is small, relatively inexpensive, easy to use in the field and powerful. This would enable the whole flight path of bees to be tracked over large areas, rather than just small parts of it.

Conclusions of medium and large scale work

- Bumble bees tended to remain loyal to one *Aconitum septentrionale* patch or its nearest neighbour, which could lead to limited pollen flow between patches
- Bumble bees appeared very loyal to forage areas within a radius of ca. 50 m. Data may be limited because of decreased likelihood of reobserving individuals at long distances from their marking point using the transect method.
- Few bumble bees crossed between meadows. Further work is needed to establish whether the forests acted as physical barriers to bee movement, or whether the restricted within-meadow movements were a result of innately-controlled bumble bee foraging strategies

Movement by bumble bees in relation to their nest

The original aim to establish foraging areas of whole nests of bees in Chapter 4 failed because of low reobservation number of marked bees in the crop (on average < 1% per session). It was assumed that the crop would provide a highly attractive resource to which the bees would be loyal. The result that most bees did not visit it was unexpected. This work emphasised the importance of nest-traffic measurement in order to estimate the number of foragers at any time, and therefore reobservation rates of marked bees. Using a time-lapse video in this work was a key methodological advance in achieving accurate nest traffic data. The few authors who have measured nest traffic previously used labour intensive manual methods. Recent development of a photocell recorder is also promising (see Dramstad 1996a). Most reports of bumble bee foraging in relation to the nest did not even mention nest traffic and based conclusions of foraging areas on a few individuals only. General agreement in the literature is that bumble bees generally forage within a few hundred metres of their nest but there has not been convincing empirical data yet to support this (Dramstad 1996b). The work in this thesis indicates that foraging areas could easily exceed previous estimates. Foragers were found at 750 m from their nests (Chapter 4). The area of a circle of 750 m radius encompasses 176 ha compared with an estimate of 12 ha resulting from a radius of 200 m from the nest.

A second methodological advance was the successful development of a mass-marking system to automatically mark foragers leaving the nest. Previously, manual marking methods were labour intensive and unreliable.

An effort was made to avoid results being affected by a common sampling artefact presented in animal dispersal study literature. The leptokurtic curve of flight distance distribution is prevalent in the pollinator movement literature (see Kearns & Inouye 1993). It results from partial sampling of an area. To overcome this, the whole area of crop was observed using equal effort for every part of the crop. A leptokurtic curve of flight distances still arose however, showing that *some* bees *do* forage close to their nest. This result may have been partly due to the crop's narrow shape but it does suggest leptokurtic curves do have some relevance in larger scale studies and are not always completely sampling artefacts. The question of where *most* of the bees foraged still remains unanswered however. This work needs to be repeated in a larger

more typical crop with nests placed in the centre to give them equal flight opportunity. It would probably be physically impossible to search the whole area, but at least mathematical correction of data to account for decreased probability of reobservation with increasing distance from nests should be considered, if using a transect method. Also, this work should be repeated using a bee species with a good reputation for being specific to a certain crop. Work currently in progress at Lincoln University is addressing this, involving the long-tongued *B. hortorum* and a long corolla-tubed variety of red clover in a larger (5 ha) crop. So far this season, very low numbers of bees have been reobserved during transect walks, but preliminary observations show that for three of the four nests, dark brown pollen loads are the main type being returned (Barron pers. comm.). This may suggest that the more specialised *B. hortorum* tends to be more specific compared with the short-tongued *B. terrestris* generalist species, and, that pollen analysis is a valuable alternative tool for deciphering movement patterns of bees. Either multivariate analysis which takes into account the many factors influencing pollinator activity, or the isolation of key factors using artificial manipulations should be considered in future work. The problem is that both approaches are logistically difficult on a large scale. This is another situation in which radar tracking as described by Riley *et al.* (1996) would be highly useful.

A recent theory proposed is that bumble bees may not prefer to forage close to their nest as is commonly assumed. Foraging far from the nest may be an evolved behaviour to avoid predation, rather than as a result of resource depletion near the nest (see Dramstad 1996b). If this is so, it poses enormous implications for the management of bumble bees for crop pollination, both in the placement of artificially-reared nests near field crops, and in the creation of habitat refuges to encourage crop pollinators. This is a crucial aspect for further study in the future. Eventually studies should also be carried out to see if it is possible to manipulate bumble bee colonies and force them to a target crop, as is done with honey bees (see Jay 1986). There have been recent suggestions that bumble bees respond to odour cues in returning pollen loads to stimulate them to search for a specific pollen type (Plowright & Laverty 1984). Scent-training, as is practised for honey bees, could be tested on bumble bees in the future.

Conclusions regarding bumble bee movement from the nest

- Spatial representations (mesh graphs and colour density plots) are useful visual aids, but results can be misleading if not presented in combination with supporting statistical analyses.
- most bumble bees (> 90%) from nests placed on the perimeter of a seemingly-rich crop resource, did not forage on it, and therefore a colony foraging area could not be established.
- Two theories to explain the non-crop specificity by bumble bees are; resource depletion near the nest or an innate spacing-out mechanism to avoid predation. Further research is needed to differentiate which is responsible.
- Some individuals were observed up to 750 m from their nest. The extent of this longer distance travel by bumble bees remains unknown because it was physically impossible to reobserve the whole crop surroundings.
- Microclimate, honey bee density, nest reproductive success, or crop flower density and distribution could not completely explain the distribution within the crop, variation in success of reobservation rate of individuals between nests, or "loss" of bumble bees from the study area on their own.
- Time-lapse video recording of nest traffic, and a new mass-marking method proved to be reliable and effective tools for providing more accurate data than in past studies.

Pollen and nectar rewards in a crop and non-crop plant species

This work in Chapter 5, an extension of Chapter 4 showed that considerable variations in pollen and nectar rewards exist between crop and non-crop species. Future work needs to establish the relative influence of these rewards on movement patterns of bees on a large scale. If non-crop specificity by bumble bees is widespread, the future of field management of introduced nests may be limited. Different plant and bumble bee species combinations should be tested in typical farmland situations. More related studies are needed on exactly how bumble bees discriminate between flower species to visit in terms of pollen and nectar rewards. Some authors suggest that nitrogen content is a key factor in pollen choice, for example.

Conclusions of pollen and nectar survey

- A weed species *L. salicaria* offered more highly concentrated nectar, and more pollen to bumble bees than the crop species *M. sativa*. This difference in rewards may help to explain the non-specificity of bumble bees from introduced nests to the crop in previous experiments. Further research is needed to match specific bumble bee nutritional requirements with pollen and nectar rewards offered by target crops.

Alternative approaches for the future for large scale movement studies

In the future alternative approaches to establish bumble bees' use of space will become important. Mark-reobservation studies do provide useful information, but as demonstrated they also have limitations. The development of techniques involving pollen analysis (Whidden 1996), genetic markers (Estoup *et al.* 1995; Kennet 1995), radioactive compounds (Lecomte & Pouvreau 1968), and radar tracking of foragers (Hornsby 1996; Riley *et al.* 1996) offer a range of promising tools for future research.

In summary, this thesis has provided data on bumble bee movement patterns in two farmland situations. It is hoped that in the future information of this type will be combined with temporal aspects of bumble bee foraging behaviour, and plant genetic data in order to evaluate and predict the efficacy of bumble bees as pollinators. Spatial data should be regarded as a valuable component in the development of management strategies for bumble bees as pollinators of both crop and wildflower species.

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Appendicies

APPENDIX 2.1

Taxonomy of bumble bee species studied in this thesis
(based on Alford 1975; Løken 1985; Prys-Jones & Corbet 1984).

Genus *Bombus* Latreille - "True bumble bees"

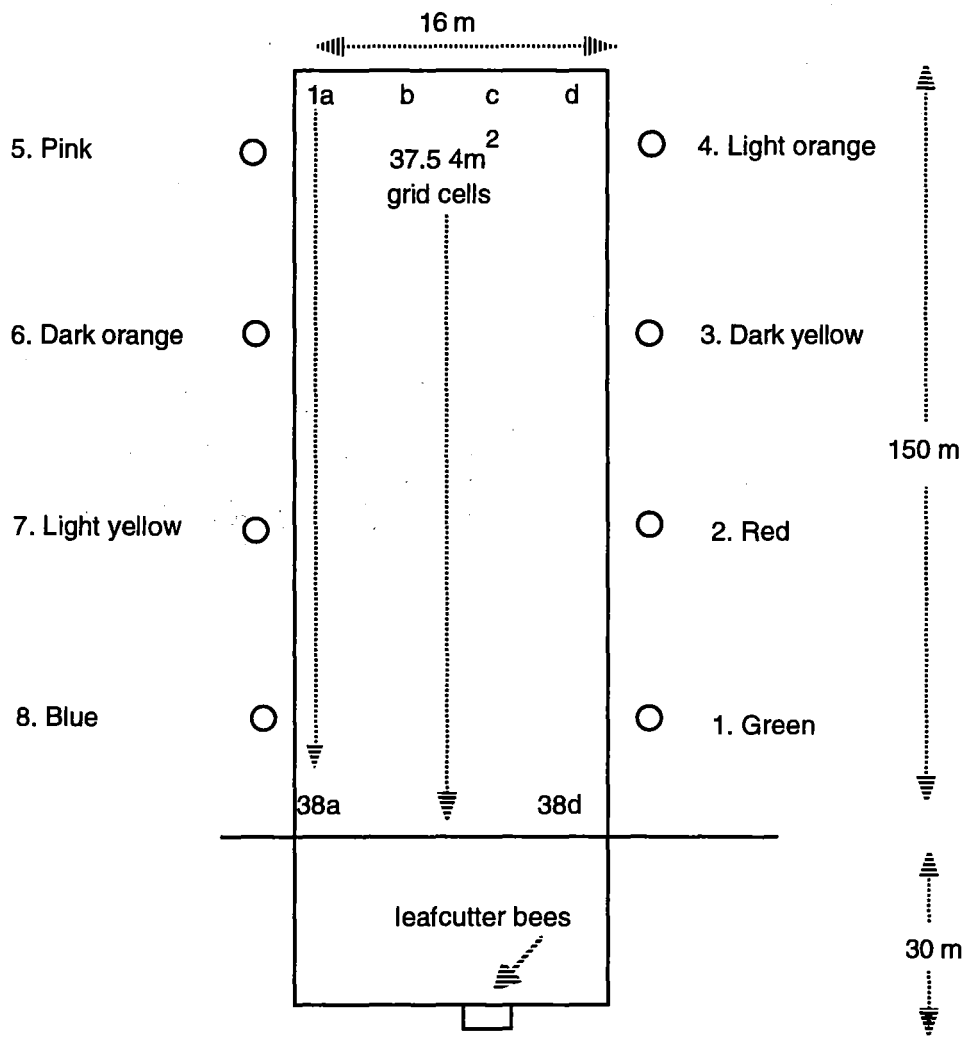
Species	Subgenus
Norwegian species:	
B. lucorum (Linnaeus)*	*Bombus* Latreille
B. terrestris (Linnaeus)*	*Bombus* Latreille
B. pratorum (Linnaeus)	*Pyrobombus* von Dalla Torre
B. lapidarius (Linnaeus)	*Melanobombus* von Dalla Torre
B. hortorum (Linnaeus)	*Megabombus* von Dalla Torre
B. ruderatus (Fabricius)	*Megabombus* von Dalla Torre
B. pascuorum (Scopoli)	*Thoracobombus* von Dalla Torre
B. subterraneus (Linnaeus)	*Subterraneobombus* Vogt
B. monticola (Smith)	*Pyrobombus* von Dalla Torre
B. hypnorum (Linnaeus)	*Bombus* Latreille
New Zealand species:	
B. terrestris (Linnaeus)	*Bombus* Latreille
B. hortorum (Linnaeus)	*Megabombus* von Dalla Torre

* indistinguishable in the field, and therefore referred to as *B. lucorum* only in the Norway-related work in this thesis.

N.B. Individuals of the "Cuckoo bumble bee" genus *Psithyrus* Lepeletier were not identified to species level in this thesis.

APPENDIX 4.1.

Sketch map of lucerne crop study site



Sketch map of lucerne crop study site (not to scale) showing location of bumble bee nests (O). Nests 1 & 8 between rows 27 & 28, nests 2 & 7 between rows19 & 20, nests 3 & 6 between rows12 & 13, nests 4 & 5 between rows 4 & 5. Nests placed 0.5 m from crop edge.

APPENDIX 4.2.

Fluorescent marking powders

Supplier: BASF New Zealand Ltd.
38 Mangere Drive
Auckland
New Zealand

subsidary of: Day Glo Colour Corporation of Nalco Chemical Company Inc.
4515 St. Clair Avenue
Cleveland, Ohio
US

Company colour description	Company code	Name used in thesis
1. Signal green	T-18-N	Green
2. Red	122-9046	Red
3. Strong yellow	2X-34	Dark yellow
4. Arc yellow	T-16	Light orange
5. Strong magenta	NX-15	Pink
6. Strong orange	NX-21	Dark orange
7. Saturn yellow	Z-17-N	Light yellow
8. Horizon blue	STX-19	Blue

APPENDIX 5.1

Methodology for the determination of nectar volumes and sugar concentrations from nectar spots on filter paper (after Dafni 1992):

A. Determination of nectar volume:

1. Spot nectar on Whatman no. 1 chromatography paper.
2. Measure the spot diameter and compare it with the calibration table (Table A.5.1) given to estimate volume. This method is limited to spots up to 12 mm in diameter.

A. Determination of sugar concentration

(i): Preparation of PABA staining solution

Solution A: 75 mg oxalic acid dissolved in 15 ml ethanol

Solution B: 150 mg PABA (p-amino benzoic acid) dissolved in 25 ml chloroform and 2 ml glacial acetic acid

(ii): Examination procedure

1. Prepare Whatman No.1 chromatography paper discs using a standard paper punch
2. Spot the nectar onto the paper discs and record its volume (the sugar spot need not fill the entire circle). Use a clean disc as a control.
3. Put each disc in a test-tube and cover with 50 μl of distilled water and allow to stand for 0.5-1 h.
4. Add 150 μl of PABA staining solution and mix well. Allow to dry. (Use a vacuum dessicator to speed up this process.)
5. When dry, heat for 10 min at 100^{o} C, until a brown colour develops
6. Redissolve the stain in 1 ml methanol:water (1:1 v/v) and transfer eluted stain into semi-micro cuvettes. This stain is stable for at least 1 day. Read in spectrophotometer at 470 nm, using the control tube as a blank.
7. Sugar quantities can be obtained from suitable calibration curves using standard solutions of known sugar content. The sucrose content should correspond approximately to that of the unknown
8. Use more than one set of standards
9. The procedure gives amounts of sugar in a known volume (i.e., weight per volume)

This method is accurate mainly in the 30-60% range.

Table A.5.1. Correlation of nectar spot size with volume on Whatman No.1 filter paper (after Dafni 1992)

Diameter (in mm)	Volume (µl)	Diameter (in mm)	Volume (µl)	Diameter (in mm)	Volume (µl)
0.1	0.01	4.1	0.650	8.1	3.3
0.2	0.02	4.2	0.700	8.2	3.4
0.3	0.03	4.3	0.750	8.3	3.5
0.4	0.04	4.4	0.800	8.4	3.6
0.5	0.05	4.5	0.850	8.5	3.7
0.6	0.06	4.6	0.900	8.6	3.8
0.7	0.07	4.7	0.950	8.7	3.9
0.8	0.08	4.8	1.000	8.8	4.0
0.9	0.09	4.9	1.050	8.9	4.1
1.0	0.10	5.0	1.100	9.0	4.2
1.1	0.11	5.1	1.160	9.1	4.31
1.2	0.12	5.2	1.220	9.2	4.42
1.3	0.13	5.3	1.280	9.3	4.53
1.4	0.14	5.4	1.340	9.4	4.64
1.5	0.15	5.5	1.400	9.5	4.75
1.6	0.16	5.6	1.460	9.6	4.86
1.7	0.17	5.7	1.520	9.7	4.97
1.8	0.18	5.8	1.580	9.8	5.08
1.9	0.19	5.9	1.640	9.9	5.19
2.0	0.20	6.0	1.700	10.0	5.30
2.1	0.215	6.1	1.765	10.1	5.45
2.2	0.230	6.2	1.830	10.2	5.60
2.3	0.245	6.3	1.895	10.3	5.75
2.4	0.260	6.4	1.960	10.4	5.90
2.5	0.275	6.5	2.025	10.5	6.05
2.6	0.290	6.6	2.090	10.6	6.20
2.7	0.305	6.7	2.155	10.7	6.35
2.8	0.320	6.8	2.220	10.8	6.50
2.9	0.335	6.9	2.285	10.9	6.65
3.0	0.350	7.0	2.350	11.0	6.80
3.1	0.375	7.1	2.435	11.1	6.99
3.2	0.400	7.2	2.520	11.2	7.18
3.3	0.425	7.3	2.605	11.3	7.37
3.4	0.450	7.4	2.690	11.4	7.56
3.5	0.475	7.5	2.775	11.5	7.75
3.6	0.500	7.6	2.860	11.6	7.94
3.7	0.525	7.7	2.945	11.7	8.13
3.8	0.550	7.8	3.030	11.8	8.32
3.9	0.575	7.9	3.115	11.9	8.51
4.0	0.600	8.0	3.200	12.0	8.70

APPENDIX 5.2

Conversion of sucrose content in nectar (% by volume) to weight (mg) per volume:

Example calculation for a flower sample containing 0.149 μl of nectar of 54.8% sucrose concentration:

1. The molecular weight of sucrose (Mr) = 342.3 g/mol
2. 54.8% x 0.149 μl = 0.08162 μl of sucrose
3. If a 50% sucrose concentrated solution has 50 g sucrose in 100 ml
then a 54.8% sucrose concentrated solution has 54.89 g sucrose in 100 ml

4. n (number of moles) = 54.8%/342.3/0.1 (volume correction) = 1.601 mol/l
If n= M/Mr, then M(mass) = nMr:
1.601 {mol/l} x 0.149 x 10^{-6} l
=2.38 x 10^{-7} mol x 342.3 g/mol
= 8.1652 x 10-3 g
=0.081652 mg sucrose per flower sample

APPENDIX 5.3

Estimation of pollen grain number per flower; method and sample calculation

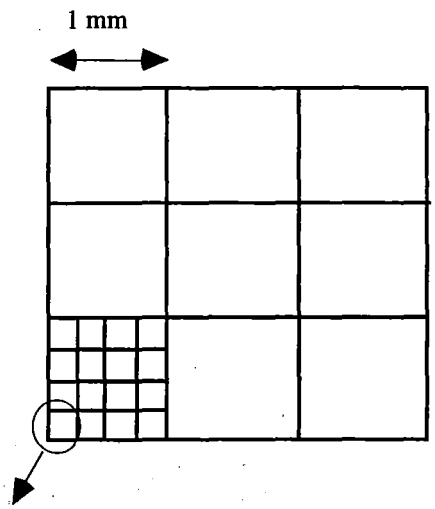
Method after Dafni (1992) and (Kearns & Inouye 1993).

1. Squash a ripe anther (just before pollen exposure) into 0.9 ml of 70% ethanol + 3 drops of 5% methylene blue dye + 4 drops of detergent to prevent grains clumping.
2. Transfer the squashed anther (carefully rinse the forceps and the blade in the droplet to prevent loss of material) into a calibrated tube and fill up to 1 ml with the same ethanol and detergent mixture. (For *M. sativa* and *L. salicaria* samples in Chapter 5 of this thesis, volumes were made up to 1.0 and 0.5 ml respectively).
3. Shake well (or stir the suspension with a vortex mixer for 60 to 90 seconds).
4. Transfer six separate samples of 1 μl (or an appropriate volume depending on the plate specifications) onto a haemacytometer plate and count pollen grains. (A disposable-tipped 20 μl dispensing pipette was convenient for use in Chapter 5 of this thesis.)
5. Calculate the average number of pollen grains per flower by making an extrapolation based on the dilution factor, number of anthers put into the sample, and number of anthers per plant.

Example from Chapter 5 of this thesis:

A haemacytometer plate (model Mod-fuchs rosenthal B. S. 748 Hawksley Cristalite) of specifications: "1/16th mm^2, 0.2 mm" was used (see explanation in sketch below).

If 22 purple loosestrife pollen grains (from a solution of 0.5 ml) were counted over all nine of the large etched squares of the haemacytometer plate shown below; then total pollen grains were calculated using the following steps;



0.25 mm x 0.25 mm surface area x 0.2 mm depth

1. If the volume (mm^2) contained in the smallest division of the etched grid (circled above)
= 0.0625 mm^2 x 0.2 mm
= 0.0125 mm^3

2. Then the volume contained on the whole etched grid = 0.0125 mm^3 x (12 x 12 squares of smallest diison)
= 1.8 mm^3
3. If 1cm^3=1 ml
then 1.8 mm^3 = 0.0018 cm^3
therefore the count plate holds a total volume of 0.0018 ml

4. To calculate total pollen grains in the 0.5 ml sample:
22 grains counted on plate x 0.5 ml/ 0.0018 ml
= 22 grains x 277.7
= 6111

5. The 22 grains in the sample came from a flower which produced 12 anthers in total, but only six were bee-available at any time. Two anthers only were sampled per flower. Therefore the count plate result was multiplied by three to estimate total bee-available pollen in the flower at any time:

6. 6111 x 3 = 18 333 grains per flower.